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(54) Title: NEW A83543 COMPOUNDS AND PROCESS FOR PRODUCTION THEREOF

(57) Abstract

New A83543 components and salts thereof, and N-demethyl derivatives are useful for the control of insects and mites. Methods for making the new A83543 components by culture of *Saccharopolyspora spinosa* NRRL 18719, NRRL 18720, NRRL 18723 are provided. Insecticidal and ectoparasiticidal compositions containing new A83543 components are also provided. Pseudoaglycones of Aare useful for the preparation of A83543 components.

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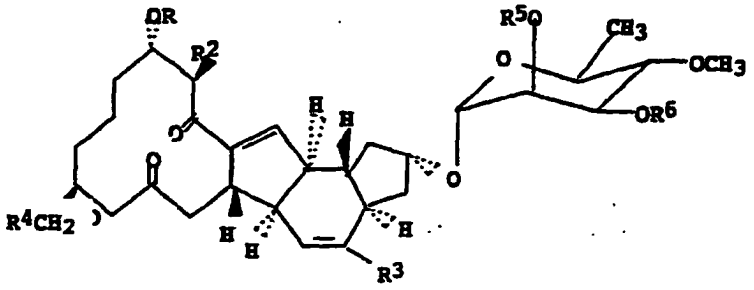
New A83543 Compounds and Process for Production Thereof

The invention relates to new compounds of fermentation product A83543.

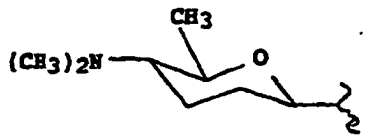
Target insects are rapidly developing resistance to synthetic insecticides, including the development of resistance to the newer pyrethroid insecticides (see Pickett (1988), Chem. Britain, 137). Therefore, new insecticides are in demand.

Fermentation product A83543, a family of related compounds produced by strains of *Saccharopolyspora spinosa*, was recently discovered and was shown to exhibit excellent insecticidal activity. A83543 and each of the compounds are useful for the control of mites and insects, particularly *Lepidoptera* and *Diptera* species.

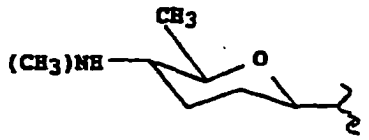
By "A83543 compounds" is meant natural compounds consisting of a 5,6,5-tricylic ring system, fused to a 12-membered macrocyclic lactone, a neutral sugar and an amino sugar (see Kirst *et al.* (1991), Tetrahedron Letters, 32:4839). The family of natural A83543 compounds includes a genus taught in EPO Application No. 0375316 and having the following general formula:



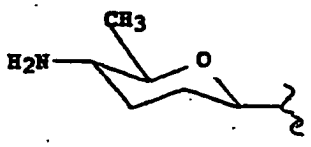
wherein R^1 is H or a group selected from



(a)



(b)



(c)

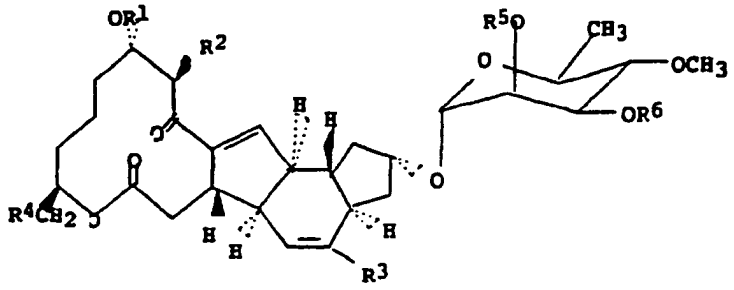
or

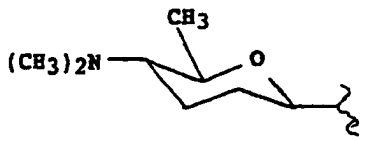
(d)

and R^2, R^4, R^3, R^5 and R^6 are hydrogen or methyl; or an acid addition salt thereof when R^1 is other than hydrogen.

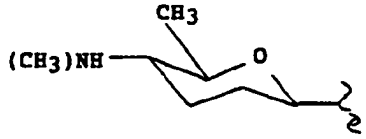
The A83543 fermentation product has been shown to comprise individual compounds A83543A, A83543B, A83543C, A83543D, A83543E, A83543F, A83543G, A83543H and A83543J and various psuedoaglycones thereof (see

European Patent Publication No. O 375 316). The structures of these individual compounds are shown below.

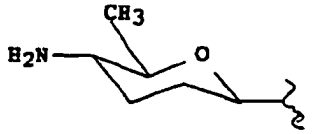




(a)

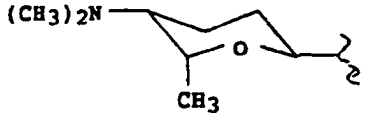


(b)



(c)

or



(d)

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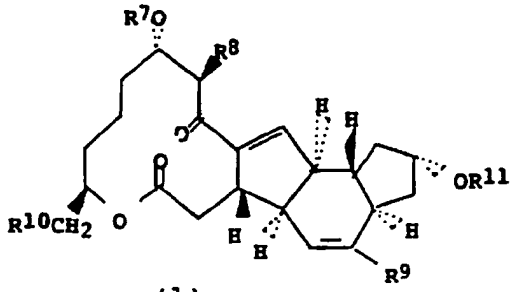
wherein R^1, R^2, R^3, R^4, R^5 and R^6 are for each compound as follows:

Structures of A83543 Compounds

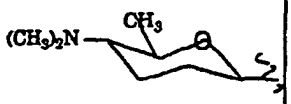
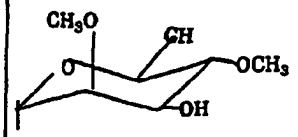
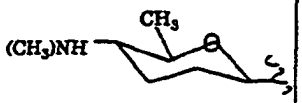
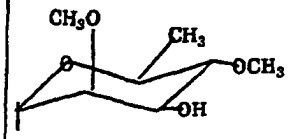
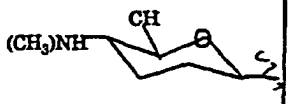
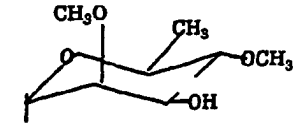
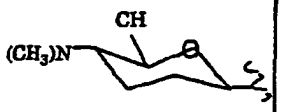
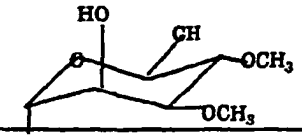
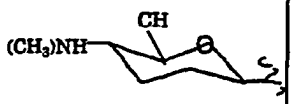
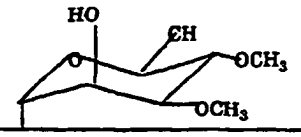
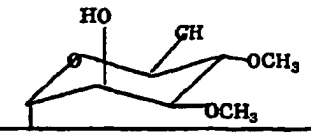
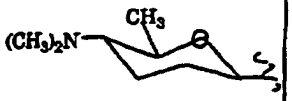
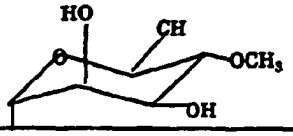
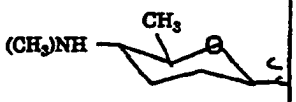
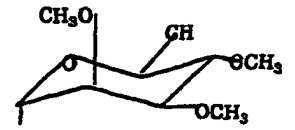
Compound	R^1	R^2	R^3	R^4	R^5	R^6
A	(a)	Me	H	Me	Me	Me
B	(b)	Me	H	Me	Me	Me
C	(c)	Me	H	Me	Me	Me
D	(a)	Me	Me	Me	Me	Me
E	(a)	Me	H	H	Me	Me
F	(a)	H	H	Me	Me	Me
G	(d)	Me	H	Me	Me	Me
H	(a)	Me	H	Me	H	Me
J	(a)	Me	H	Me	Me	H
PsaA1	H	Me	H	Me	Me	Me
PsaD1	H	Me	Me	Me	Me	Me
PsaE1	H	Me	H	H	Me	Me
PsaF1	H	H	H	Me	Me	Me
PsaH1	H	Me	H	Me	H	Me
PsaJ1	H	Me	H	Me	Me	H
PsaL1	H	Me	Me	Me	Me	H

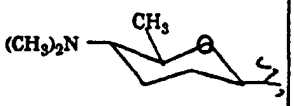
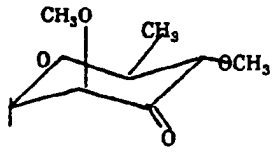
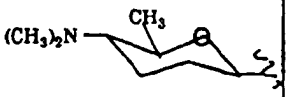
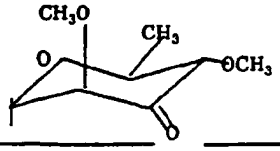
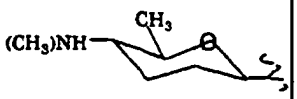
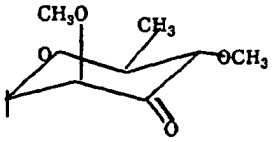
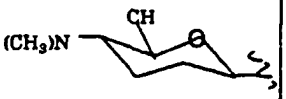
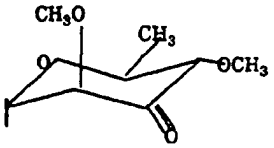
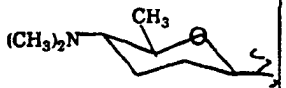
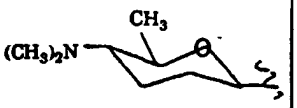
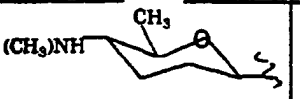
The present invention is directed to compounds of Formula 1:

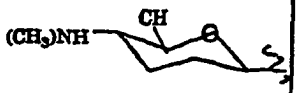
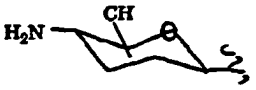
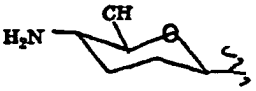
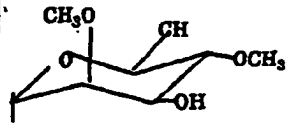


(1)

wherein R^7, R^8, R^9 and R^{10} are each individually as follows:

Compound	R^7	R^8	R^9	R^{10}	R^{11}
Compound 1 (A83543L)		Me	Me	Me	
Compound 2 (A83543M)		Me	H	Me	
Compound 3 (A83543N)		Me	Me	Me	
Compound 4 (A83543Q)		Me	Me	Me	
Compound 5 (A83543R)		Me	H	Me	
Compound 6 (A83543S)		Me	H	H	
Compound 7 (A83543T)		Me	H	Me	
Compound 8 (N-demethyl A83543D)		Me	Me	Me	

Compound	R^7	R^8	R^9	R^{10}	R^{11}
Compound 9		Me	H	Me	
Compound 10		Me	Me	Me	
Compound 11		Me	H	Me	
Compound 12		Me	Me	Me	
Compound 13 (A83543 PsaA2)		Me	H	Me	H
Compound 14 (A83543 PsaD2)		Me	Me	Me	H
Compound 15 (A83543 PsaB2)		Me	H	Me	H

Compound	R^7	R^8	R^9	R^{10}	R^{11}
Compound 16 (A83543 PsaN2)		Me	Me	Me	H
Compound 17 (A83543 AgA)	H	Me	H	Me	H
Compound 18 (A83543 AgD)	H	Me	Me	Me	H
Compound 19 (A83543 AgE)	H	Me	H	H	H
Compound 20 (A83543 AgF)	H	H	H	Me	H
Compound 21 (A83543 PsaC2)		Me	H	Me	H
Compound 22		Me	Me	Me	H
Compound 23 (A83543 PsaL2)	H	Me	Me	Me	

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The present invention is also directed to insecticidal and miticidal compositions and methods for reducing the populations of insects and mites using Compounds 1-8, which are compounds of Formula 1 wherein R^7 is other than hydrogen.

Another aspect of the present invention is a process for producing Compounds 1-7, novel natural components of A83543 fermentation product, which comprises cultivating *S. spinosa* strains, or mutants thereof, capable of producing Compounds 1-7, in a suitable culture medium, under submerged aerobic fermentation conditions, until a recoverable amount of any one of Compounds 1-7 is produced. Compounds 1-7 can be isolated and purified as described hereinafter.

This invention further provides a biologically purified culture of newly discovered *S. spinosa* strains NRRL 18719 (A83543.6), NRRL 18720 (A83543.7) and NRRL 18823 (A83543.9).

The chemical structures of Compounds 1-7 were determined by spectrometric methods, including nuclear magnetic resonance spectroscopy (NMR), and ultraviolet spectroscopy (UV), and by comparison to the known A83543 compounds (see Kirst, *et al.* (1991), *supra*). The following paragraphs describe the physical and spectral properties of Compounds 1-7:

A83543L:

A83543L has the following characteristics:

Molecular weight: 731

Empirical formula: $C_{41}H_{65}NO_{10}$

UV (EtOH): 244 nm (ε=10,362)

MS (FAB): (M+H) m/z 732

Table I summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543L (in d_6-acetone).

Table I. ^{1}H and ^{13}C NMR data of A83543L in acetone-d_6

Position	^{13}C	^{1}H*
1	172.59	--
2	34.32	3.07/2.42
3	48.39	2.91
4	42.75	3.45
5	123.29	5.53
6	137.19	--
7	45.33	2.18
8	35.61	2.01/1.45
9	76.62	4.32
10	38.59	2.36/1.38
11	46.99	1.03
12	49.99	2.77
13	148.51	7.02
14	145.11	--
15	203.09	--

Table I. (Continued)

Position	^{13}C	$^{1}H^{*}$
16	48.43	3.30
17	80.91	3.55
18	35.04	1.55
19	22.50	1.79/1.19
20	30.89	1.53
21	76.81	4.45
22	29.11	1.49
23	9.55	0.81
24	16.29	1.13
6-CH_3	20.85	1.74
1'	96.37	4.86
2'	82.44	3.33
3'	72.33	3.73
4'	84.59	2.95
5'	68.34	3.50
6'	18.31	1.20
2'-OCH_3	59.05	3.44
4'-OCH_3	60.74	3.51
1"	104.06	4.46
2"	31.90	1.93/1.39
3"	18.74	1.83/1.52
4"	65.97	2.12
5"	74.04	3.57
6"	19.39	1.21
$N(CH_3)_2$	40.97	2.21

* Values were taken from a heteronuclear one bond 2D correlation spectrum.

A83543M:

A83543M has the following characteristics:

Molecular weight: 703
Empirical formula: $C_{39}H_{61}NO_{10}$
UV (EtOH): 244 nm (ε=10,240)
MS (FAB): (M+H) m/z 704

Table II summarizes the ^1H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543M (in d_6-acetone).

Table II. ^1H and ^{13}C NMR data of A83543M in acetone-d_6

Position	^{13}C	$^1H^*$
1	172.65	--
2	34.53	3.08/2.44
3	48.80	2.94
4	42.36	3.50
5	129.80	5.87
6	130.34	5.91
7	42.05	2.14
8	37.21	1.97/1.36
9	77.04	4.34
10	38.30	2.36/1.36
11	47.12	0.94
12	50.44	2.87
13	148.34	7.05
14	144.93	--
15	203.08	--

Table II. (Continued)

Position	^{13}C	^{1}H*
16	48.36	3.31
17	81.22	3.55
18	35.15	1.52
19	22.38	1.78/1.17
20	31.09	1.50
21	76.82	4.66
22	29.11	1.48
23	9.57	0.80
24	16.44	1.13
1'	96.46	4.84
2'	82.50	3.31
3'	72.23	3.72
4'	84.61	2.94
5'	68.40	3.48
6'	18.34	1.19
2'-OCH_3	59.08	3.44
4'-OCH_3	60.72	3.50
1"	104.15	4.48
2"	31.78	1.88/1.42
3"	29.11	2.11/1.23
4"	61.86	2.02
5"	76.55	3.27
6"	19.34	1.22
$N(CH_3)_2$	34.16	2.34

* Values were taken from a heteronuclear one bond 2D correlation spectrum.

A83543N:

A83543N has the following characteristics:

Molecular weight: 717
Empirical formula: $C_{40}H_{63}NO_{10}$
UV (EtOH): 244 nm (ε=10,446)
MS (FAB): (M+H) m/z 718

Table III summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543N (in d_6-acetone).

Table III. ^{1}H and ^{13}C NMR data of A83543N in acetone-d_6

Position	^{13}C	^{1}H*
1	172.65	--
2	34.41	3.06/2.43
3	50.00	2.90
4	42.85	3.45
5	123.38	5.55
6	137.25	--
7	45.39	2.18
8	35.67	2.01/1.46
9	76.78	4.32
10	38.65	2.37/1.40
11	47.07	1.03
12	50.07	2.78
13	148.41	7.03
14	145.17	--
15	203.14	--

Table III. (Continued)

Position	^{13}C	$^{1}H^{*}$
16	48.44	3.31
17	81.14	3.56
18	35.13	1.51
19	22.44	1.81/1.20
20	31.05	1.51
21	76.78	4.65
22	29.11	1.48
23	9.56	0.81
24	16.41	1.12
6-CH_3	20.82	1.73
1'	96.51	4.86
2'	82.51	3.32
3'	72.24	3.73
4'	84.62	2.95
5'	68.41	3.50
6'	18.34	1.18
2'-OCH_3	59.09	3.43
4'-OCH_3	60.71	3.51
1"	104.13	4.48
2"	31.78	1.87/1.40
3"	29.11	2.11/1.23
4"	61.88	2.00
5"	76.58	3.26
6"	19.33	1.22
$N(CH_3)_2$	34.18	2.34

* Values were taken from a heteronuclear one bond 2D correlation spectrum.

A83543Q:

A83543Q has the following characteristics:

Molecular weight: 731
Empirical formula: $C_{41}H_{65}NO_{10}$
UV (EtOH): 244 nm (ε=10,492)
MS (FAB): (M+H) m/z 732

Table IV summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543Q (in d_6-acetone).

Table IV. ^{1}H and ^{13}C NMR data of A83543Q in acetone-d_6

Position	^{13}C	^{1}H*
1	172.64	--
2	34.30	3.08/2.44
3	48.94	2.91
4	42.77	3.44
5	123.24	5.55
6	137.65	--
6-CH_3	20.82	1.74
7	45.32	2.18
8	35.47	2.00/1.45
9	76.35	4.32
10	38.52	2.36/1.39
11	46.96	1.05
12	49.98	2.79
13	148.53	7.04
14	145.12	--
15	203.12	--

Table IV. (Continued)

Position	^{13}C	$^{1}H^{*}$
16	48.44	3.31
17	80.88	3.53
18	35.05	1.50
19	22.53	1.81/1.18
20	30.87	1.53
21	76.84	4.65
22	29.12	1.48
23	9.53	0.81
24	16.24	1.12
1'	99.51	4.75
2'	68.44	3.94
3'	82.52	3.33
4'	82.62	3.06
5'	68.33	3.55
6'	18.19	1.20
3'-OCH_3	56.81	3.39
4'-OCH_3	60.71	3.46
1"	104.06	4.47
2"	31.90	1.94/1.39
3"	18.72	1.81/1.49
4"	65.98	2.12
5"	74.05	3.56
6"	19.39	1.21
$N(CH_3)_2$	40.95	2.21

* Values were taken from a heteronuclear one bond 2D correlation spectrum.

A83543R:

A83543R has the following characteristics:

Molecular weight: 703

Empirical formula: $C_{39}H_{61}NO_{10}$

UV (EtOH): 245 nm (ε=10,991)

MS (FAB): (M+H) m/z 704

Table V summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543R (in acetone-d_6).

Table V. ^{1}H and ^{13}C NMR data of A83543R in acetone-d_6

Position	^{13}C	$^{1}H^{*}$
1	172.63	-
2	34.41	30.8/2.46
3	48.71	2.95
4	42.23	3.51
5	129.67	5.87
6	130.29	5.91
7	42.04	2.16
8	37.02	1.95/1.36
9	76.64	4.33
10	38.19	2.37/1.38
11	47.01	0.93
12	50.33	2.87
13	148.43	7.06
14	144.84	-
15	203.08	-

Table V. (Continued)

Position	^{13}C	$^{1}H^{*}$
16	48.32	3.31
17	80.99	3.56
18	35.06	1.52
19	22.41	1.79/1.19
20	30.91	1.59/1.46
21	76.83	4.66
22	29.06	1.48
23	9.55	0.81
24	16.31	1.13
1'	99.51	4.74
2'	68.31	3.93
3'	82.48	3.33
4'	82.59	3.07
5'	68.31	3.53
6'	18.18	1.19
3'-OCH_3	56.80	3.39
4'-OCH_3	60.70	3.47
1"	104.16	4.48
2"	31.70	1.88/1.42
3"	29.06	2.11/1.23
4"	61.82	1.98
5"	76.52	3.26
6"	19.30	1.22
$NHCH_3$	34.18	2.35

* Values were taken from a heteronuclear one bond 2D correlation spectrum.

A83543S:

A83543S has the following characteristics:

Molecular weight: 703
Empirical formula: $C_{39}H_{61}NO_{10}$
UV (EtOH): 244 nm (ε=9,697)
MS (FAB): (M+H) m/z 704

Table VI summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543S (in acetone-d_6).

Table VI. ^{1}H and ^{13}C NMR data of A83543S in acetone-d_6

Position	^{13}C	$^{1}H^{*}$
1	172.39	--
2	34.86	3.06/2.40
3	48.79	2.95
4	42.03	3.43
5	129.66	5.86
6	130.32	5.91
7	41.99	2.16
8	37.04	1.97/1.38
9	76.65	4.33
10	38.20	2.35/1.38
11	47.08	0.93
12	50.31	2.86
13	148.37	7.05
14	144.74	--
15	203.01	--

Table VI. (Continued)

Position	^{13}C	^{1}H*
16	47.98	3.34
17	81.16	3.56
18	34.90	1.61/1.52
19	22.25	1.80/1.17
20	33.57	1.50
21	72.97	4.68
22	21.62	1.12
24	16.44	1.13
1'	99.51	4.73
2'	68.41	3.93
3'	82.51	3.33
4'	82.59	3.06
5'	68.31	3.53
6'	18.19	1.19
3'-OCH_3	56.81	3.38
4'-OCH_3	60.71	3.47
1"	104.05	4.47
2"	31.92	1.94/1.41
3"	18.71	1.83/1.52
4"	65.95	2.13
5"	74.03	3.56
6"	19.39	1.21
$N(CH_3)_2$	40.96	2.21

* Values were taken from a heteronuclear one bond 2D correlation spectrum

A83543T:

A83543T has the following characteristics:

Molecular weight: 703

Empirical formula: $C_{39}H_{61}NO_{10}$

UV (EtOH): 245 nm (ε=13,082)

MS (FAB): (M+H) m/z 704

Table VII summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for A83543T (in acetone-d_6).

Table VII. ^{1}H and ^{13}C NMR data of A83543T in acetone-d_6

Position	^{13}C*	^{1}H*
1	172.65	--
2	34.25	3.11/2.49
3	48.50	2.97
4	41.92	3.54
5	129.69	5.91
6	130.31	5.94
7	42.01	2.18
8	37.03	1.98/1.39
9	76.44	4.36
10	38.18	2.39/1.39
11	47.32	0.96
12	50.14	2.89
13	148.36	7.09
14	144.85	--
15	203.12	--

Table VII. (Continued)

Position	^{13}C*	^{1}H*
16	48.22	3.35
17	80.83	3.57
18	34.99	1.57/1.51
19	22.34	1.82/1.22
20	30.83	1.58/1.49
21	76.72	4.69
22	29.04	1.52
23	9.29	0.84
24	16.14	1.16
1'	99.46	4.75
2'	72.61	3.71
3'	72.61	3.69
4'	84.12	3.05
5'	68.22	3.57
6'	18.18	1.24
4'-OCH_3	60.55	3.60
1"	104.55	4.50
2"	31.81	1.97/1.43
3"	18.59	1.86/1.55
4"	65.76	2.15
5"	73.98	3.60
6"	19.24	1.24
$N(CH_3)_2$	40.95	2.25

* Values were taken from 1D or inverse 2D one bond configuration.

Another aspect of the present invention is the chemical demethylation of natural factor A83543D to produce N-demethyl A83543D. Similarly, A83543M and A83543N may be prepared from A83543J and A83543L.

The N-demethyl derivatives are prepared by reacting a natural factor in the presence of between one and thirteen equivalents of iodine and a suitable base such as sodium acetate. The reaction is carried out in a polar organic solvent, such as methanol, or a mixture of a polar organic solvent and water, such as aqueous methanol. The reaction is preferably carried out at a temperature of from between 30°C to 90°C for between 2 to 6 hours, at a pH of between 8 and 10.

N-demethyl A83543D:

N-demethyl A83543D has the following characteristics:

UV (EtOH): 244 nm (ε = 9400)

Table VIII summarizes the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data for N-demethyl A83543D (in acetone-d_6).

Table VIII. ^{1}H and ^{13}C NMR of N-demethyl A83543D, in acetone-d_6.

Position	^{13}C	$^{1}H^{*}$
1	172.62	--
2	34.38	3.09/2.47
3	48.97	2.91
4	45.38	2.19
5	123.38	5.55
6	137.20	--
7	42.80	3.47
8	35.59	2.02/1.45
9	76.78	4.66
10	38.64	2.36/1.37
11	47.03	1.03
12	50.02	2.78
13	148.36	7.03
14	145.17	--
15	203.08	--
16	48.43	3.31
17	81.08	3.55
18	31.01	1.52
19	22.45	1.79/1.19
20	35.11	1.52
21	76.70*	4.33

Table VIII. (Continued)

Position	^{13}C	^{1}H*
22	29.11	1.48
23	9.56	0.81
24	16.39	1.13
1'	97.10	4.84
2'	78.35	3.55
3'	82.56	3.41
4'	83.13	3.02
5'	68.69	3.50
6'	18.27	1.19
2'-OCH	57.32	3.41
3'-OCH	59.04	3.44
4'-OCH	60.70	3.47
1"	104.10	4.47
2"	31.79	1.86/1.42
3"	29.23	2.09/1.48
4"	61.94	2.00
5"	76.60*	3.25
6"	19.34	1.22
6CH	20.84	1.75
NHCH	34.31	2.34

* Values were taken from a heteronuclear one bond 2D correlation spectrum.

Compounds 1-8 can react to form various acid addition salts. Representative suitable salts include those salts formed by standard reactions with both organic and inorganic acids such as, for example, sulfuric, hydrochloric, phosphoric, acetic, succinic, citric, lactic, maelic, fumaric, cholic, pamoic, mucic, glutamic, camphoric, glutaric, glycolic, phthalic,

tartaric, formic, lauric, stearic, salicyclic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic, and like acids. These salts are useful, for example, in separating and purifying Compounds 1-8. In addition, some of the salt forms may have increased water solubility. These salts are prepared using standard procedures for salt preparation.

The present invention is also directed to the preparation of pseudoaglycones by the reaction of natural components with an acid to remove the amino sugar. Suitable acids include hydrochloric and sulfuric, preferably sulfuric. The reaction is preferably carried out in a polar organic solvent, a mixture of a polar organic solvent and water. Suitable organic solvents include methanol, THF, acetonitrile and dioxane. The preferred solvents for the transformation are a mixture of methanol and water. The reaction may be carried out at a temperature from about 25°C to about 95°C, preferably at 80°C.

The pseudoaglycones of the present invention are produced by the following scheme:

Scheme A

A83543J→Compound 9→Compound 13→Compound 17
A83543PsaA2→A83543AgA
A83543L→Compound 10→Compound 14→Compound 18
A83543PsaD2→A83543AgD
A83543M→Compound 11→Compound 15→Compound 19
A83543PsaB2→A83543AgA
A83543N→Compound 12→Compound 16→Compound 20
A83543PsaN2→A83543AgD

Accordingly, in another one of its aspects, the invention provides a process for producing A83543AgA, A83543AgD, A83543AgE, or A83543AgF, which comprises

(a) hydrolyzing A83543A, A83543B, A83543C, A83543G, A83543H, A83543J, A83543PsaA1, A83543PsaA2, A83543PsaH1, A83543PsaJ1, A83543PsaB2, or A83543PsaC2 to produce A83543Aga; or

(b) hydrolyzing A83543PsaD2 or A83543PsaN2 to produce A83543AgD; or

(c) hydrolyzing A83543E or A83543PsaE1 to produce A83543AgE; or

(d) hydrolyzing A83543F or A83543PsaF1 to produce A83543AgF.

The pseudoaglycones are useful as starting materials for the preparation of new A83543 compounds, for example, the pseudoaglycone may be glycosylated at the hydroxyl group where the amino sugar was present. This glycosylation may be carried out by chemical synthesis or by microbial bioconversion. More specifically, A83543PsaL1 may be bioconverted to A83543L

and A83543N by culturing any of the known A83543-producing strains in the presence of A83543PsaL1.

Compounds 1-7, natural A83543 components, are generally produced by culturing a suitable A83543-producing strain of *S. spinosa* sp. nov. under submerged aerobic conditions in a desired culture medium, until a recoverable amount of a natural factor is produced. Compounds 1-7 can be recovered using various isolation and purification procedures which are understood in the art.

For convenience in the discussions which follow, two known A83543A-producing strains have been given the following designations: A83543.1 and A83543.4 (see EPO No. 0 375 316); as discussed below these strains have been used to develop new strains. Two new A83543J-producing strains have been given the designation A83543.6 and A83543.7; components A83543L, A83543M and A83543N are produced by A83543.6 and A83543.7. Finally, a new A83543Q-producing strain has been given the designation A83543.9; components A83543Q, A83543R, A83543S and AZ83543T are produced by A83543.9. Cultures A83543.1, A83543.4, A83543.6, A83543.7 and A83543.9 have been deposited and made a part of the stock culture collection of the Midwest Area Regional Research Center, Agricultural Research Service, United States Department of Agriculture, from which they are available to the public under the following accession numbers:

NRRL No.	Strain No.
18395	A83543.1
18538	A83543.4
18719	A83543.6
18720	A83543.7
18823	A83543.9

Culture A83543.1 was obtained by chemical mutation of culture A83543, which was isolated from a soil sample collected in the Virgin Islands (see Mertz and Yao (1990), Int'l J. of Systematic Bacteriology, 40:34). Cultures A83543.4 and A83543.6 were derived from culture A83543.1 by chemically-induced mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. Culture A83543.7 and A83543.9 were derived from A83543.4 by chemically-induced mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. The following data show that these distinct isolates are all strains of *S.spinosa* and have very few cultural, morphological or biochemical differences. Except for differences in the production of the A83543 components, these isolates appear similar to the parent culture.

Cultural Characteristics

Cultures A83543.1, A83543.4, A83543.6 A83543.7 and A83543.9 were grown on twelve agar plating media and compared for growth, reverse color, aerial hyphae production, spore mass color, and soluble pigment production. No significant differences were observed on any of the media used. The cultures grew well on both complex and defined media. Aerial hyphae were produced on most of the media used. The aerial spore mass color was predominantly white, and the reverse side was yellow to yellow-brown. No distinctive pigmentation was

present; however, a soluble brown pigment was released into some media. These cultural characteristics are the same as presented in the original taxonomic description of A83543.1 (see Mertz and Yao (1990), *supra*).

Morphological Characteristics

No significant differences were observed between any of the strains compared. Well-formed aerial hyphae, which were segmented into long chains of spores arranged as hooks and open loops, were present on most of the media. Spirals were also observed, but they were short and incomplete. The general morphology was rectus-flexibilis. Aerial hyphae of each of the strains had a distinctive bead-like appearance, with many empty spaces in the spore chain. This feature demonstrated that a spore sheath encased the spore chain, which is a distinctive feature of the genus *Saccharopolyspora*.

Physiological Characteristics

Fatty acid analyses from each of the strains were compared. Cells were grown for 96 hours at 28°C in trypticase soy broth (Difco Laboratories, Detroit, MI). Fatty acid methyl esters were analyzed by gas-liquid chromatography with a model 5898A computer-controlled gas-liquid chromatography system (Hewlett-Packard Co., Palo Alto, CA) (see Miller and Berger, "Bacterial Identification by Gas Chromatography of Whole Cell Fatty Acids," Hewlett-Packard Application Note 228-41. These results are presented in Table IX).

TABLE IX. Percentage Fatty Acid Composition of A83543 Strains

Fatty Acid	A83543.1	A83543.4	A83543.6	A83543.7	A83543.9
15:0 ISO	15.95	22.47	16.49	17.00	17.42
16:0 ISO	28.71	22.00	25.76	27.39	24.34
16:1 Cis 9	--	1.35	--	--	0.92
15:0 ISO 2OH	2.67	2.02	3.87	3.95	1.78
16:0	1.20	0.69	0.63	0.60	0.36
17:1 ISO F[1]	5.52	8.62	7.54	5.51	8.72
17:0 Iso	13.55	20.67	16.40	13.89	19.43
17:0 Anteiso	8.39	3.94	4.69	5.18	5.52
17:1 B	4.14	3.97	4.65	6.68	4.61
17:1 C	2.52	2.88	4.90	5.53	3.02
17:0	4.26	1.49	3.13	3.84	1.67
16:1 2OH	1.87	1.52	1.93	0.92	2.17
18:1 Iso F	6.55	4.16	5.82	6.00	5.74
18:1 Cis 9	0.34	1.03	0.64	0.63	0.84

[1]F, B and C indicate double bond positions or configurations that are unknown.

Principal-component analysis is a branch of multivariate statistics that deals with internal relationships of a set of variables. In this analysis, the greatest amount of variance within the original data or test results is expressed as principal components (see Alderson, "The Application and Relevance of Nonheirarchic Methods in Bacterial Taxonomy", in Computer-Assisted Bacterial Systematics 227 (1985)). A plot showing scatter or variability can be constructed. Relationships can be evaluated by examining the variance, and a microbial population characterized. A two-dimensional principal component plot from the fatty acid analyses of strains A83543.1, A83543.4, A83543.6, A83543.7 and A83543.9 is shown in Figure 1. The values refer to the degrees of separation between the strains involved. The differences between the strains represent strain differences.

As is the case with other organisms, the characteristics of the A83543A-producing, A83543J-producing and A83543Q-producing strains are subject to variation. Thus, mutants of these strains may be obtained by physical and chemical methods known in the art. For example, other strains may be obtained by treatment with chemicals such as N-methyl-N'-nitro-N-nitrosoguanidine.

One aspect of the present invention is the production of Compounds 1-3 by culturing an A83543-producing strain of *S. spinosa*, selected from the group consisting of NRRL 18719 and NRRL 18720, or an A83543J-producing mutant thereof, in a suitable culture medium. An "A83543J-producing mutant" is a natural or induced mutant derived from *S. spinosa* NRRL NRRL 18719 or NRRL 18720 which is capable of producing recoverable amounts

of A83543J (as well as A83543L, A83543M or A83543N). Similarly, Compounds 4-7 are produced by culturing *S. spinosa* strain NRRL 18823, or an A83543Q-producing mutant thereof, in a suitable culture medium. An "A83543Q-producing mutant" is a strain derived from *S. spinosa* NRRL 18823 which is capable of producing recoverable amounts of A83543Q (as well as A83543R, A83543S or A83543T). Strain NRRL 18823 produces A83543 components containing α-3,4-di-O-methylrhamnose. The biosynthetic mechanism for methylating the 2-hydroxy group of rhamnose is defective in this new strain.

After production, Compounds 1-7 may be separated from the culture medium using various isolation and purification procedures which are well understood in the art. For economy in production, optimal yield, and ease of product isolation, certain culture media are preferred. For example, preferred carbon sources in large-scale fermentation are glucose and methyl oleate, although ribose, xylose, fructose, galactose, mannose, mannitol, soluble starch, potato dextrin, oils such as soybean oil and the like can also be used. Preferred nitrogen sources are cottonseed flour, peptonized milk and corn steep liquor, although fish meal, digested soybean meal, yeast extract, enzyme-hydrolyzed casein, beef extract, and the like can also be used. Among the nutrient inorganic salts which can be incorporated in the culture media are the customary soluble salts capable of yielding zinc, sodium, magnesium, calcium, ammonium, chloride, carbonate, sulfate, nitrate and like ions. Essential trace elements necessary for the growth and development of the organism should also be included in the culture medium. Such trace elements commonly occur as impurities in

other substituents of the medium in amounts sufficient to meet the growth requirements of the organism.

Usually, if foaming is a problem, small amounts (i.e., 0.2 ml/L) of an antifoam agent such as polypropylene glycol may be added to large-scale fermentation media. In the case of the A83543-producing cultures, however, conventional defoamers inhibit A83543 production. Foaming can be controlled by including soybean oil or PLURONIC L-101 (BASF, Parsippany, NJ) in the medium (1-3%). Additional oil may be added if foaming develops.

For production of substantial quantities the natural factors, submerged aerobic fermentation in stirred bioreactors is preferred; however, small quantities of natural factors may be obtained by shake-flask culture. Because of the time lag in production commonly associated with inoculation of large bioreactors with the spore form of the organism, it is preferable to use a vegetative inoculum. The vegetative inoculum is prepared by inoculating a small volume of culture medium from a stock culture preserved in liquid nitrogen to obtain a fresh, actively growing culture of the organism. The vegetative inoculum is then transferred to a larger bioreactor. The vegetative inoculum medium can be the same as that used for larger fermentations, but other media are also suitable.

Compounds 1-3 are produced by A83543J-producing strains and Compounds 4-7 are produced by A83543Q-producing strains when grown at temperatures between about 24° and about 33°C. Optimum temperatures for production appear to be about 28-30°C.

As is customary in submerged aerobic culture processes, sterile air is blown into the vessel from the bottom while the medium is stirred with conventional turbine impellors. In general, the aeration rate and agitation rate should be sufficient to maintain the level of dissolved oxygen at or above 80% of air saturation, preferably above 70%, with an internal vessel pressure of about 0.34 atmospheres.

Production of Compounds 1-7 can be followed during the fermentation by testing extracts of the broth. A preferred method for following the production is analysis of the broth extracts by high performance liquid chromatography (HPLC). Suitable systems for analysis are described in Examples 1 and 7.

Following the production in shake flasks or in stirred reactors, Compounds 1-7 can be recovered from the fermentation medium by methods used in the art. The compounds produced during fermentation of the A83543J-producing or A83543Q-producing strains occur in both the mycelia and the broth. Compounds 1-7 are lipophilic; when a substantial amount of oil is used in the fermentation, whole broth extraction is more efficient. If only small amounts of oil are used, the major portion of the Compounds 1-7 is present in the mycelia. In that case, more efficient recovery of Compounds 1-7 is accomplished by initially filtering the medium to separate the broth from the mycelial mass (the biomass).

Compounds 1-7 can be recovered from the biomass by a variety of techniques. A suitable technique involves washing the separated biomass with water to remove remaining broth, mixing the biomass with a polar solvent in which Compounds 1-7 is soluble, e.g.,

methanol or acetone, separating and concentrating the solvent, extracting the concentrate with a non-polar solvent and/or adsorbing it onto a reverse-phase silica gel adsorbent, such as reverse phase C_8 or C_{18} resin, or a high porous polymer such as HP-20 or HP-20SS (Mitsubishi Chemical Industries Co., Ltd., Japan). The active material is eluted from the adsorbent with a suitable solvent such as, for example, acetonitrile:methanol mixtures, optionally containing small amounts of THF.

A preferred technique for isolating Compounds 1-7 from the biomass involves adding an equal volume of acetone to the whole broth, filtering the mixture in a ceramic filter to remove the biomass, and extracting the filtrate with ethyl acetate. The ethyl acetate extract is concentrated *in vacuo* to remove the acetone, and the aqueous layer is separated from the organic layer. The ethyl acetate solution is further concentrated *in vacuo*, and the concentrate is extracted with dilute aqueous acid (pH 3). Compounds 1-7 may be further purified by chromatography as described herein.

A more preferred technique for isolating Compounds 1-7 from the biomass involves adding an equal volume of acetone to the whole broth, filtering the mixture in a ceramic filter to remove the biomass, and adjusting the pH of the filtrate to about pH 9 to about pH 13. This solution is applied to HP-20SS (Mitsubishi Chemical Industries Co., Ltd., Japan) and the column washed with a mixture of methanol, acetonitrile, and water (1:1:2). Any one of Compounds 1-7 may be eluted with a 95:5 mixture of methanol/acetonitrile (1:1) and aqueous 0.1% ammonium acetate (pH 8.1). The fractions containing Compounds 1-7 are combined and lyophilized.

Compounds 1-7 may be further purified by chromatography as described herein.

Alternatively, the culture solids, including medium constituents and mycelium, can be used without extraction or separation, but preferably after removal of water, as a source of Compounds 1-7. For example, after production of Compounds 1-7, the whole fermentation broth can be dried by lyophilization, by drum-drying, or by azeotropic distillation and drying. The dried broth can then be used directly, for example, by mixing it directly into feed premix or into formulations for sprays and powders.

Compounds 1-8 are capable of inhibiting insects or mites. The term "inhibiting an insect or mite" refers to a decrease in the number of living insects or mites or to a decrease in the number of viable insect or mite eggs. Generally, an amount in the range from about 1 to about 1,000 ppm (or 0.01 to 1 kg/a) of Compounds 1-8 is used.

More specifically, Compounds 1-8 show activity against beet armyworm and tobacco budworm, which are members of the insect order *Lepidoptera*. Other typical members of this order are southern armyworm, codling moth, cutworms, clothes moths, indian meal moth, leaf rollers, corn ear worm, cotton bollworm, European corn borer, imported cabbage worm, cabbage looper, pink bollworm, bagworms, eastern tent caterpillar, sod webworm, and fall armyworm.

Compounds 1-8 also show activity against leaf hoppers, which is a member of the insect order *Homoptera*. Other members of this order include cotton aphid, plant hoppers, pear psylla, apple sucker, scale insects, whiteflies, and spittle bugs, as well as a number of other host-specific aphid species.

In addition, Compounds 1-8 show activity against stable flies, blowflies, and mosquitoes, which are members of the insect order *Diptera*. Another typical member of this order is the common house fly.

Compounds 1-8 also show activity against two-spotted spider mites, which is a member of the insect order *Acarina*. Other typical members of this order include mange mite, scab mite, sheep scab mite, chicken

mite, scalyleg mite, depluming mite, and dog follicle mite.

Compounds 1-8 are used in a method of inhibiting an insect or mite population which comprises applying to a locus of the insect or mite an effective insect- or mite-inactivating amount of at least one compound selected from Compounds 1-8. In one preferred embodiment, the present invention is directed to a method for inhibiting a susceptible insect of the order *Lepidoptera* which comprises applying to a plant an effective insect-inactivating amount of at least one compound selected from Compounds 1-8 in accordance with the present invention. Another preferred embodiment of the invention is directed to a method of inhibiting biting flies of the order *Diptera* in animals which comprises administering an effective pest-inhibiting amount of at least one compound selected from Compounds 1-8 orally, parenterally, or topically to the animal. In another preferred embodiment, the present invention is directed to a method for inhibiting a susceptible insect of the order *Homoptera* which comprises applying to a plant an effective insect-inactivating amount of at least one compound selected from Compounds 1-8. Another preferred embodiment of the invention is directed to a method of inhibiting mites of the order *Acarina* which comprises applying to the locus of the mite a mite-inactivating amount of at least one compound selected from Compounds 1-8.

Mite/Insect Screen

Compounds 1-8 were tested for miticidal and insecticidal activity in the following mite/insect screen. Each test compound was formulated by dissolving

the compound in an acetone-alcohol (1:1) mixture containing 23 g of TOXIMUL R (sulfonate/nonionic emulsifier blend) and 13 g of TOXIMUL S (sulfonate/-nonionic emulsifier blend) per liter. These mixtures were then diluted with water to give the indicated concentrations.

Two-spotted spider mites and cotton aphids were introduced on squash cotyledons and allowed to establish on both leaf surfaces. The leaves were then sprayed with 5 ml of test solutions using a DeVilbiss atomizing sprayer at 10 psi. Both surfaces of the leaves were covered until run off and then allowed to dry for one hour. After standard exposure periods percent mortality was evaluated. Additional insects were evaluated using similar formulations and evaluation procedures. The results are reported in Table X. The following abbreviations are used:

Abbreviation	Pest	Scientific Name
ALH	Aster Leafhopper	*Macrosteles fascifrons*
BAW	Beet Armyworm	*Spodoptera exiqua*
CA	Cotton Aphid	*Aphis gossypii Glover*
CBW	Cotton Bollworm	*Heliothis zea*
GECR	German Cockroach	*Blattella germanica*
NEM	Rootknot Nematode	*Meliiodyne spp.*
SAW	Southern armyworm	*Spodoptera eridinia*
SCRW	Southern Corn Rootworm	*Diabrotica undecimpunctata howardi*
TBW	Tobacco Budworm	*Heliothis virescens*
TSSM	Two-spotted Spider Mite	*Tetranychus urticae*

Table X. Activity in Insect/Mite Screen

Pest	rate[a]	perc	% Inhibition[b] Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4	Cmpd 5	Cmpd 6	Cmpd 7
ALH	200	24 hr	100	60	100	100	0	60	20
	400	24 hr	100	100	100	100	0	100	100
BAW	200	6 day	100	0	90	100	0	100	0
	400	6 day	100	0	100	100	0	100	0
CA	200	4-5 day	0	0	0	0	0	0	0
	400	4-5 day	0	0	0	0	0	0	0
GECR	200	7 day	40	0	0	0	0	0	0
	400	7 day	0	0	0	0	0	0	0
	200	21 day	100	20	0	0	20	0	0
	400	21 day	100	40	0	0	0	20	0
NEM	200	11 day	0	0	0	0	0	0	0
	400	11 day	0	0	0	0	0	0	0
SCRW	200	11 day	0	0	0	0	0	0	0
	400	11 day	0	0	0	0	0	0	0
TBW	200	6 day	100	70	100	100	63	26	0
	400	6 day	100	100	100	100	88	53	0
TSSM	200	4-5 day	0	0	0	70	85	0	0
	400	4-5 day	0	0	0	100	90	0	0

[a] rate ppm
[b] % inhibition as a mean of single replicate tests
[c] exposure period.

Compound 8 (N-demethyl-A83543D) was also tested and found to be active against SAW, SCRW and TSSM.

Compounds 1-8 were evaluated using the following assay to determine the LD_{50} against neonate tobacco budworm (*Heliothis virescens*). A petri dish (100mm x 20mm) is inverted and the lid lined with a #1 qualitative filter paper. Ten neonate larvae are placed in each dish and a 1 ml test solution is pipetted onto the insects. The petri dish bottom is then placed on the lid to contain the larvae. At 1 hr. after treatment, a small piece of *Heliothis* diet (modified slurry, Southland Products, Lake Village, AR) is added to each dish. The mortality is evaluated at 24 and 48 hours. The tests were run in triplicate. The results are shown in Table XI.

Table XI. Activity Against Neonate Tobacco Budworm

Compound	LD_{50} (ppm)[a]
1 (A83543L)	26.0
2 (A83543M)	22.6
3 (A83543N)	40.0
4 (A83543Q)	0.39
5 (A83543R)	14.5
6 (A83543S)	53.0
7 (A83543T)	)64.0
8 (N-demethyl-A83543D)	5.8

[a] mean of two tests

Insecticidal Compositions

Compounds 1-8 of this invention are applied in the form of compositions which comprise an insect- or mite- inactivating amount of any one of Compounds 1-8 in a phytologically acceptable inert carrier. Any one of Compounds 1-8 may be present as a single compound, a

mixture of two or more compounds, a mixture of at least one compound selected from Compounds 1-8 or a mixture of at least one compound selected from Compounds 1-8 together with the dried portion of the fermentation medium in which it is produced.

Compositions are prepared according to procedures and formula which are conventional in the agricultural chemical art, but which are novel and important because of the presence of one or more of the compounds of this invention. The compositions are either concentrated formulations which are dispersed in water for application or dust or granular formulations which are applied without further treatment.

The dispersions in which the compound or crude dried material are applied are most often aqueous suspensions or emulsions prepared from concentrated formulations of compounds or crude material, such as water-soluble, water-suspendible, or emulsifiable formulations are either solids (usually known as wettable powders) or liquids (usually known as emulsifiable concentrates or aqueous suspensions).

Wettable powders, which may be compacted to form water dispersible granules, comprise an intimate mixture of the active compound, an inert carrier, and surfactants. The concentration of the active compound is usually from about 1% to about 90% by weight. The inert carrier is usually chosen from among attapulgite clays, the montmorillonite clays, the diatomaceous earths or the purified silicates.

Effective surfactants, comprising from about 0.5% to about 10% of the wettable powder are found among

the sulfonated lignins, the condensed naphthalene-sulfonates, the napthalene-sulfonates, the alkyl-benzenesulfonates, the alkylsulfates, and nonionic surfactants such as ethylene oxide adducts of alkylphenols.

Emulsifiable concentrates of the compounds comprise a convenient concentration of a compound, such as from about 50 to about 500 grams per liter of liquid, equivalent to about 10% to about 50%, dissolved in an inert carrier which is either a water-miscible solvent or mixture of a water-immiscible organic solvent and emulsifiers. Useful organic solvents include aromatics, especially the xylenes, and petroleum fractions, especially high-boiling naphthlenic and olefinic portions of petroleum such as heavy or aromatic naphtha. Other organic solvents may also be used, such as the terpenic solvents, including rosin derivatives, aliphatic ketones such as cyclohexanone, and complex alcohols such as 2-ethoxyethanol. Suitable emulsifiers for emulsifiable concentrates are chosen from conventional nonionic surfactants, such as those mentioned above.

Aqueous suspensions comprise suspensions of water-insoluble compounds of this invention dispersed in an aqueous vehicle at a concentration in the range from about 5% to about 50% by weight. The suspensions are prepared by finely grinding the compound, and vigorously mixing it into a vehicle comprised of water and surfactants chosen from the same types discussed above. Inert ingredients, such as inorganic salts and synthetic or natural gums may also be added to increase the density and viscosity of the aqueous vehicle. It is often most effective to grind and mix the compound at

the same time by preparing the aqueous mixture and homogenizing it in an implement such as a sand mill, ball mill, or piston-type homogenizer.

Compounds 1-8 may also be applied as granular compositions, which are particularly useful for applications to the soil. Granular compositions usually contain from about 0.5% to about 10% by weight of at least one of Compounds 1-8, dispersed in an inert carrier which consists entirely or in large part of clay or a similar inexpensive substance. Such compositions are usually prepared by dissolving the compound in a suitable solvent and applying it to a granular carrier which has been pre-formed to the appropriate particle size in the range of from about 0.5 to 3 mm. Such compositions may also be formulated by making a dough or paste of the carrier, drying the combined mixture of the active ingredient in the dough or paste, and crushing the dried composition to obtain the desired granular particle size.

Dusts containing the compound are prepared by intimately mixing the compound in powdered form with a suitable dust agricultural carrier, such as kaolin clay, ground volcanic rock, and the like. Dusts can suitably contain from about 1% to about 10% of at least one compound selected from Compounds 1-8.

It is equally practical, when desirable for any reason, to apply the compound in the form of a solution in an appropriate organic solvent, usually a bland petroleum oil, such as the spray oils, which are widely used in agricultural chemistry.

Insecticides and miticides are usually applied in the form of a dispersion of the active ingredient in a liquid carrier. The most widely used carrier is water.

Compounds 1-8 can also be applied in the form of an aerosol composition. In such compositions the active compound is dissolved in an inert carrier, which is a pressure-generating propellent mixture. The aerosol composition is packaged in a container from which the mixture is dispersed through an atomizing valve. Propellent mixtures comprise either low-boiling halocarbons, which may be mixed with organic solvents, or aqueous suspensions pressurized with inert gases or gaseous hydrocarbons.

The amount of compound to be applied to the loci of insects and mites is not critical and can readily be determined by those skilled in the art in view of the examples provided. In general, concentrations of from about 10 ppm to about 5,000 ppm of at least one compound selected from Compounds 1-8 are expected to provide good control. With many of the compounds, concentrations of from about 100 ppm to about 1,000 ppm will suffice. For field crops, such as soybeans and cotton, a suitable application rate for the compounds is about 0.01 kg/ha to about 1 kg/ha, typically applied in a 5 gal/A to 50 gal/A of spray formulation.

The locus to which at least one compound selected from Compounds 1-8 is applied can be any locus inhabited by an insect or mite, for example, vegetable crops, fruit and nut trees, grape vines and ornamental plants. Because of the unique ability of mite eggs to

resist toxicant action, repeated applications may be desirable to control newly emerged larvae, as is true of other known acaricides.

Ectoparasiticide Activity

Tables XII and XIII summarize *in vitro* studies using compounds of the present invention against members of the insect order *Diptera*.

Table XII. Activity Against Blowfly Larvae

Compound	Activity rate (ppm)	% mortality
A83543L	5	90
A83543Q	10	100
	5	90
A83543R	10	100
	5	100

Table XIII. Activity Against Adult Stablefly

Compound	Activity rate (ppm)	% mortality 24 hrs	48 hrs
A83543L	5	70	100
A83543M	5	60	100
A83543N	5	20	90
A83543Q	10	90	100
	5	50	90
A83543R	10	90	100
	5	70	100

Ectoparasiticidal Methods

The ectoparasiticidal method of this invention is carried out by administering at least one of

Compounds 1-8 to host animals to control insect and *Acarina* parasites. Administration to the animal may be by the dermal, oral or parenteral routes.

Parasitic insects and *Acarina* parasites include species that are bloodsucking as well as flesh eating and are parasitic during all of their life cycle or only part of their life cycle, such as only the larval or only the adult stage. Representative species include the following:

horse fly	*Tabanus spp.*
stable fly	*Stomoxys calcitrans*
black fly	*Simulium spp.*
horse sucking louse	*Haematopinus asini*
mange mite	*Sarcoptes scabiei*
scab mite	*Psoroptes equi*
horn fly	*Haematobia irritans*
cattle biting louse	*Bovicola bovis*
shortnosed cattle louse	*Haematopinus eurysternus*
longnosed cattle louse	*Linoqnathus vituli*
tsetse fly	*Glossina spp.*
cattle follicle mite	*Demodex bovis*
cattle tick	*Boophilus microplus and B. decoloratus*
Gulf Coast tick	*Amblyomma maculatum*
Lone Star tick	*Amblyomma americanum*
ear tick	*Otobius meqnini*
Rocky Mountain wood tick	*Dermacentor andersoni*
screw-worm fly	*Cochliomyia hominivorax*
assassin bug	*Reduvius spp.*
mosquito	*Culiseta inornata*
brown ear tick	*Rhipicephalus appendiculatus*

African red tick	*Rhipicephalus evertsi*
bont tick	*Amblyomma sp.*
bont legged tick	*Hyalomma sp.*
hog louse	*Haematopinus suis*
chigoe	*Tunqa penetrans*
body louse	*Haematopinus ovillus*
foot louse	*Linoqnathus pedalis*
sheep ked	*Melophaqus ovinus*
sheep scab mite	*Psoroptes ovis*
greenbottle fly	*Phaenicia sericata*
black blow fly	*Phormia reqina*
secondary screw-worm	*Cochliomyia macellaria*
sheep blow fly	*Phaenicia cuprina*
bed bug	*Cimex lectularius*
Southern chicken flea	*Echidnophaqa qallinacea*
fowl tick	*Arqas persicus*
chicken mite	*Dermanyssus qallinae*
scalyleg mite	*Knemidokoptes mutans*
depluming mite	*Knemidokoptes qallinae*
dog follicle mite	*Demodex canis*
dog flea	*Ctenocephalis canis*
American dog tick	*Dermacentor variabilis*
brown dog tick	*Rhipicephalus sanguineus*

The method of the invention may be used to protect economic and companion animals from ecto-parasites. For example, Compounds 1-8 may beneficially be administered to horses, cattle, sheep, pigs, goats, dogs, cats and the like, as well as to exotic animals such as camels, llamas, deer and other species which are commonly referred to as wild animals. The compound may also beneficially be administered to poultry and other birds, such as turkeys, chickens, ducks and the like.

Preferably, the method is applied to economic animals, such as cattle and sheep.

Ectoparasiticidal Compositions

This invention also relates to methods and compositions for controlling a population of insect ectoparasites which consume blood of a host animal. These compositions may be used to protect economic, companion and wild animals from ectoparasites. The compositions may also beneficially be administered to poultry and other birds.

The rate, timing and manner of effective application will vary widely with the identity of the parasite, the degree of parasital attack and other factors. Applications can be made periodically over the entire life span of the host, or for only peak season of parasitic attack. In general ectoparasite control is obtained with topical application of liquid formulations containing from about 0.0005 to about 95% of at least one compound selected from Compounds 1-8, preferably up to 5%, and most preferably up to 1% of at least one compound selected from Compounds 1-8. Effective parasite control is achieved at an administration rate from about 5 to about 100 mg/kg.

Compounds 1-8 are applied to host animals by conventional veterinary practices. Usually the compounds are formulated into ectoparasiticidal compositions which comprise at least one compound selected from Compounds 1-8 and a physiologically-acceptable carrier. For example, liquid compositions may be simply sprayed on the animals for which ectoparasiticidal control is desired. The animals may

also treat themselves by such devices as back rubbers which may contain at least one compound selected from Compounds 1-8 and a cloth, for example, which the animal may walk against in contact. Dip tanks are also employed to administer the active agent to the host animal.

Oral administration may be performed by mixing the compound in the animals' feed or drinking water, or by administering dosage forms such as tablets, capsules, boluses or implants. Percutaneous administration is conveniently accomplished by subcutaneous, intraperitoneal, and intravenous injection of an injectible formulation.

Compounds 1-8 can be formulated for oral administration in the usual forms, such as drenches, tablets or capsules. Such compositions, of course, require orally-acceptable inert carriers. The compounds can also be formulated as an injectible solution or suspension, for subcutaneous, dermal, intraruminal, intraperitoneal, intramuscular, or intravenous injection. In some applications the compounds are conveniently formulated as one component of a standard animal feed. In this embodiment it is usual to formulate the present compound first as a premix in which the compound is dispersed in a liquid or particulate solid carrier. The premix can contain from about 2 to about 250 g of at least one compound selected from Compounds 1-8 per pound of mix. The premix is in turn formulated into the ultimate feed by conventional mixing.

Because ectoparasitic attack generally takes place during a substantial portion of the host animal's

life span, it is preferred to administer the compounds of the present invention in a form to provide sustained release over a period of time. Conventional procedures include the use of a matrix which physically inhibits dissolution, where the matrix is a waxy semi-solid, such as the vegetable waxes, or a high molecular weight polyethylene glycol. A good way to administer the compounds is by means of a sustained-action bolus, such as those of Laby, U.S. Patent No. 4,251,506 and Simpson, British Patent No. 2,059,767. For such a bolus the compound would be encapsulated in a polymeric matrix such as that of Nevin, U.S. Patent No. 4,273,920. Sustained release of the compounds of the present invention can also be achieved by the use of an implant such as from a silicone-containing rubber.

In order to illustrate more fully the operation of this invention, the following examples are provided:

Example 1

Assay Method for Compound 1 (A83543L), Compound 2 (A83543M) and Compound 3 (A83543N).

The following analytical high performance liquid chromatography (HPLC) method is useful for monitoring a fermentation for the production of Compound 1 (A83543L), Compound 2 (A83543M) and Compound 3 (A83543N) and other A83543 components:

A sample of the whole broth is diluted with three volumes of acetonitrile to extract the components from the mycelia. The resulting solution is then filtered through a 0.45 micron PTFE filter to remove particulate matter prior to injection into the HPLC assay system. A solution of purified A83543A at a

concentration of 100 mg/ml in methanol is used as an external standard for the assay and peak areas of all A83543 components are related back to this calibration standard to determine concentrations of individual components.

HPLC System:

Column Support: 4.6 x 100-mm column, ODS-AQ, 5µ spherical particles, 120Å pore (YMC, Inc., Morris Plains, NJ)

Mobile Phase: $CH_3CN/MeOH/H_2O$ (40/40/20) containing 0.05% ammonium acetate

Flow Rate: 3 ml/min

Detection: UV at 250 nm

Retention Times:

A83543A	9.1 min
A83543J	5.7 min
A83543L	7.3 min
A83543M	2.6 min
A83543N	3.3 min

Example 2

Preparation of A83543J, Compound 1 (A83543L), Compound 2 (A83543M) and Compound 3 (A83543N) with Culture A83543.6

A. Shake-flask Fermentation

The culture *Saccharopolyspora spinosa* NRRL 18719, either as a lyophilized pellet or as a suspension maintained in liquid nitrogen, was used to inoculate a vegetative medium having the following composition:

Vegetative Medium

Ingredient	Amount (g)
Trypticase broth*	30
Yeast extract	3
$MgSO_4 \cdot 7H_2O$	2
Glucose	5
Maltose	4
Deionized water	q.s. 1 L

Autoclave 30 min at 120°C

* Baltimore Biological Laboratories, Cockeysville, MD

Slants or plates can be prepared by adding 2.5% agar to the vegetative medium. The inoculated slant is incubated at 30°C for 10 to 14 days. The mature slant culture is scraped with a sterile tool to loosen the spores and to remove and macerate the mycelial mat. One-fourth of the loosened spores and culture growth thus obtained is used to inoculate 50 ml of a first-stage vegetative medium. Alternatively, the first-stage medium may be inoculated from a liquid nitrogen ampoule.

When the culture is maintained in liquid nitrogen, ampoules are prepared by homogenizing a vegetative culture (48-72 hours incubation, 30°C), diluting 1:1 (volume:volume) with a sterile suspending agent, and dispensing into sterile tubes (1.5 ml/tube). The suspending agent contains lactose (100 g), glycerol (200 ml), and deionized water (q.s. to 1 L).

A liquid nitrogen ampoule is used to inoculate 100 ml of vegetative medium in 500-ml Erlenmeyer flasks (or 50 ml of medium in 250-ml flasks). The cultures are incubated at 30°C for 48 hours on a shaker orbiting in a two-inch (5.08 cm) circle at 260 rpm.

The incubated culture (10% v/v inoculum) is used to inoculate 50 ml or 100 ml, dependent on the size of the Erlenmeyer flask, of a production medium having the following composition:

Production Medium

Ingredient	Amount (g)
Glucose	80
Peptonized milk*	20
Cottonseed flour**	30
Corn steep liquor	10
$CaCO_3$ (tech. grade)	5
Methyl oleate	30***
Tap water	q.s. to 1 L

pH adjusted to pH 7.0 with 1N NaOH, sterilized 40 min. at 120°C

* Peptonized Milk Nutrient, Sheffield Products, Norwich, NY

** Proflo, Traders Protein, Memphis, TN

***The amount of methyl oleate was 30 ml

The inoculated production medium is incubated in 250-ml or 500-ml Erlenmeyer flasks at 30°C for 7 to 10 days on a shaker orbiting in a two-inch circle at 260 rpm.

B. Stirred Reactor Fermentation

In order to provide a larger volume of inoculum, 10 ml of incubated first stage medium, prepared as described in Example 2, Section A, is used to inoculate 400 ml of a second-stage vegetative medium having the same composition as that of the first-stage medium. This second-stage vegetative medium is incubated in a 2 L wide-mouth Erlenmeyer flask for about 48 hours at 30°C on a shaker orbiting in a two-inch

circle at 260 rpm. Incubated second-stage vegetative medium (2 L) thus prepared is used to inoculate 80 to 115 liters of sterile production medium, prepared as described in Example 2, Section A.

The inoculated production medium is allowed to ferment in a 165 L stirred bioreactor for 7 days to 10 days at a temperature of 30°C. The air-flow and agitator speed in the stirred vessel are computer controlled to maintain a dissolved oxygen level at or above 60% to about 80% of air saturation.

Example 3

Preparation of A83543J, A83543L, A83543M, and A83543N with Culture A83543.7.

The culture *Saccharopolyspora spinosa* NRRL 18720 may be used as described in Example 2 to prepare A83543J, A83543L, A83543M, and A83543N.

Example 4

Isolation of A83543J, A83543L, A83543M, and A83543N

Fermentation broth (105 L), prepared as described in Example 2, was adjusted to pH 10 (initially pH 6.8) by adding 5N NaOH. The resulting mixture was filtered through a ceramic filter. The filtrate was discarded, a mixture of acetone and water (1:1, 50 L) was added to the mycelial solids, and the resulting mixture was filtered. A second mixture of acetone and water (1:1, 50 L) was added to the mycelial solids, and the pH of the resulting mixture was adjusted to pH 3.0 with 25% sulfuric acid. The resulting mixture was

filtered, and a third mixture of acetone and water (1:1 50 L) was added to the mycelial solid. The resulting mixture was filtered and the acidic filtrates were combined.

The combined filtrates were extracted with heptane (10 L). The phases were separated and the aqueous phase added to a second portion of heptane (10 L). The pH of the resulting mixture was adjusted to pH 10 with 5N NaOH. The resulting emulsion was diluted with 50 L of water. The phases were separated and the aqueous phase extracted with a third portion of heptane (10 L). The phases were separated and the second and third heptane extracts were combined and concentrated to a volume of about 4 liters. Upon standing, the concentrate separated into 3 phases: aqueous, emulsion, and organic. The organic phase was lyophilized to give 15.29 g of crude product.

The crude product was dissolved in methanol (500 ml), filtered, and concentrated to dryness *in vacuo*. The residue was dissolved in a second portion of methanol (20 ml) and applied to a column of LH-20 SEPHADEX (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ, 7.5 cm x 46 cm), eluting with methanol and collecting 25 ml fractions. Using the HPLC system described in Example 1, the fractions were analyzed to determine which fractions contained the compounds of interest. Fractions 18-50 were combined and concentrated to dryness.

The residue was dissolved in a mixture of ethanol, acetonitrile, and water (5:5:1) and chromatographed in 1 ml portions on a preparative reverse-phase HPLC column (Rainin DYNAMAX-60A, C18, 41.4

mm x 300 mm, 8 mm particles, 60Å pore, Woburn, MA). The column was eluted with a mixture of methanol, acetonitrile and water (87.5:87.5:25) with ammonium acetate added to a final concentration of 0.1% (pH 7.6). The fractions were analyzed using an HPLC system, similar to that as described in Example 1, combining like fractions and concentrating to give three semi-pure concentrates A, B, and C.

Semi-pure concentrate C was rechromatographed on the system described in the preceding paragraph, loading 200 ml on each of 10 runs. The fractions from each of the runs were combined and concentrated to give preparations C1 and C2. Preparation C2 was chromatographed a third time; however, water was used in place of the 0.1% ammonium acetate (desalting step). Fractions containing A83543L in at least 99.5% HPLC purity were combined and concentrated. The residue was crystallized from ethanol/water (1:1) to give 2.4 g of A83543L.

Preparation C1 and semi-pure concentrate B were combined and desalted as described in the preceding paragraph (12 x 200 ml runs); however, the desired compound was eluted with a mixture of methanol, acetonitrile, and water (11:11:3). The fractions containing A83543J in at least 99.5% HPLC purity were combined and concentrated. The residue was dissolved in hot t-butanol and lyophilized to give 4.3 g of A83543J.

Semi-pure concentrate A was chromatographed as described above, except the desired compounds were eluted with a mixture of methanol, acetonitrile, and water (37.5:37.5:25), with ammonium acetate added to final concentration of 0.1%. The fractions from each of

the runs (4) were combined and concentrated to give preparations A1, A2, and A3.

Preparation A1 was chromatographed using the column described above; however, the column was eluted with a mixture of methanol, acetonitrile, and water (2:2:1). Fractions containing A83543M in at least 99.5% HPLC purity were combined and concentrated. The residue was dissolved in t-butanol and lyophilized to give 136 mg of A83543M.

Preparation A2 was chromatographed and processed as described in the preceding paragraph to give 71 mg of A83543N.

Example 5

Synthesis of A83543M (Compound 2)

A83543J (105.4 mg, 0.15 mmol) and sodium acetate trihydrate (144.6 mg, 1.06 mmol) were added to a mixture of methanol and pH 9 buffer solution (Fisher Scientific, Lexington, MA). The resulting suspension was heated to 47°C, and then iodine (46.6 mg, .18 mol) was added in one portion. After approximately 10 min., the solution became homogeneous. After four hours at 47°C, the reaction was added to a 5% sodium thiosulfate solution. The resulting colorless aqueous mixture was extracted with methylene chloride. The methylene chloride extracts were combined, washed with brine, and dried over K_2CO_3. The dried methylene chloride solution was evaporated to dryness *in vacuo* to give 57.3 mg of A83543M as a pale yellow glass (54% yield).

Example 6

Synthesis of A83543N (Compound 3)

Using a procedure similar to that described in Example 5, A83543L (102.5 mg) was chemically converted to A83543N (66.5 mg).

Example 7

Assay Method for A83543Q (Compound 4), A83543R (Compound 5), A83543S (Compound 6) and A83543T (Compound 7)

The following analytical high performance liquid chromatography (HPLC) method is useful for monitoring a fermentation for the production of A83543Q, A83543R, A83543S, A83543T and other A83543 components:

A sample of the whole broth is diluted with three volumes of acetonitrile to extract the components from the mycelia. The resulting solution is then filtered through a 0.45 micron polytetrafluoroethylene (PTFE) filter to remove particulate matter prior to injection into the HPLC assay system. A solution of purified A83543A at a concentration of 1 mg/ml in methanol is used as an external standard for the assay and peak areas of all A83543 components are related back to this calibration standard to determine concentrations of individual components.

HPLC System:

Column Support: 4.6 x 100-mm column, ODS-AQ, 5μ spherical particles, 120Å pore (YMC, Inc., Morris Plains, NJ)

Mobile Phase: $CH_3CN/MeOH/H_2O$ (37.5/37.5/25) containing 0.05% ammonium acetate

Flow Rate: 2 ml/min

Detection: UV at 250 nm

Retention Times:

A83543A	14.97 min
A83543Q	11.82 min
A83543R	4.52 min
A83543S	6.50 min
A83543T	5.97 min
A83543H	8.50 min

Example 8

Preparation of A83543Q, A83543R, A83543S and A83543T with Culture A83543.9

A. Shake-flask Fermentation

The culture *S. spinosa* NRRL 18823, either as a lyophilized pellet or as a suspension maintained in liquid nitrogen, was used to inoculate a vegetative medium having the following composition:

Vegetative Medium 1

Ingredient	Amount(g)
Trypticase Broth*	30
Yeast extract	3
$MgSO_4 \cdot 7H_2O$	2
Glucose	5
Deionized water	q.s. 1 L

Autoclave 30 min at 120°C

* Baltimore Biological Laboratories, Cockeysville, MD

The first-stage medium may be inoculated from a liquid nitrogen ampoule. Such ampoules are prepared by homogenizing a vegetative culture (48-72 hours incubation, 30°C) diluting 1:1 (volume:volume) with a

sterile suspending agent, and dispensing into sterile tubes (1.5 ml/tube). The suspending agent contains lactose (100 g), glycerol (200) ml, and deionized water (q.s. to 1 L).

A liquid nitrogen ampoule is used to inoculate 100 ml of vegetative medium in 250-ml wide-mouthed Erlenmeyer flasks. The cultures are incubated at 30°C to 32°C for 48 hours on a shaker orbiting in a two-inch (5.08 cm) circle at 250 rpm.

The incubated culture (5% v/v inoculum) is used to inoculate 50 ml Erlenmeyer flask, of a production medium having the following composition:

Production Medium

Ingredient	Amount (g)
Glucose	80
Peptonized milk*	20
Cottonseed flour**	30
Corn steep liquor	10
$CaCO_3$ (tech. grade)	5
Methyl oleate	30***
Tap water	q.s. to 1 L

pH adjusted to pH 7.0 with 1N NaOH, sterilized 40 min. at 120°C

* Peptonized Milk Nutrient, Sheffield Products, Norwich, NY

** Proflo, Traders Protein, Memphis, TN

***The amount of methyl oleate was 30 ml

The inoculated production medium is incubated in 250-ml Erlenmeyer flasks at 30°C for 7 days on a shaker orbiting in a two-inch circle at 250 rpm.

B. Stirred Reactor Fermentation

In order to provide a larger volume of inoculum, 10 ml of incubated first stage medium, prepared as described in Example 8, Section A, is used to inoculate 400 ml of a second-stage vegetative medium having the same composition as that of the first-stage medium. This second-stage vegetative medium is incubated in a 2 L wide-mouth Erlenmeyer flask for about 48 hours at 30°C on a shaker orbiting in a two-inch circle at 260 rpm. Incubated second-stage vegetative medium (2 L) thus prepared is used to inoculate 80 to 115 liters of sterile production medium, prepared as described in Example 8, Section A.

The inoculated production medium is allowed to ferment in a 165 L stirred bioreactor for 7 days to 10 days at a temperature of 30°C. The air-flow and agitator speed in the stirred vessel are computer controlled to maintain a dissolved oxygen level at or above 60% to about 80% of air saturation.

Example 9

Isolation of A83543Q, A83543R, A83543S and A83543T from A83543.9

Fermentation broth (100 L; harvest titer A83543H, 303 µg/ml, A83543Q, 50 µg/ml), prepared as described in Example 8, was refrigerated two days prior to processing. Acetone (100 L) was added to the whole broth after adjusting the pH to 3.0 with 5N HCl. The resulting mixture was filtered through a ceramic filter to give filtrate (170 L) which was held over the weekend under refrigeration. The broth/acetone filtrate was adjusted to pH 13 and refiltered through the ceramic filter prior to loading onto a steel column (10L) containing HP-20SS resin (Mitsubishi Chemical Industries, Ltd., Japan) at a flow rate of 1 L/minute. The column was eluted at a flow rate of 1 L/minute with a gradient mixed from solvent "A" (0.1% NH_4OAc, adjusted to pH 8.1 with NH_4OH) and solvent "B" (CH_3CN - CH_3OH 1:1), collecting 4 L fractions. The pumping system was programmed to generate a gradient from 0 to 50% B in one minute, followed by a gradient from 50 to 100% B in 90 minutes, followed by isocratic delivery of 100% B for an additional 15 minutes. HPLC analysis (described in Example 7) indicated that fraction 17 (4 L), contained predominantly component R with additional more polar materials and a small amount of components T and H; fractions 18-22 contained predominantly component H with lesser amounts of components R and Q and small amounts of component S and more polar materials; fractions 23-24 contained components H and Q. HPLC analysis of the pools suggested the following total quantities;

component H, 23.0 g; component Q, 3.4 g; component R, 2.0 g; component S, 0.2 g; component T, 0.2 g.

Example 10

Recovery of A83543Q, A83543R, AS83543S, and A83543T from a Q-producing strain

Fermentation broth (85 L; harvest titer A83543H, 302 µg/ml, A83543Q, 44 µg/ml), prepared as Q-producing strain, was refrigerated overnight prior to processing. Acetone (90 L) was added to the whole broth after adjusting the pH to 3.0 with 5N HCl. The resulting mixture was filtered through a ceramic filter to give filtrate (176 L) which was held over the weekend under refrigeration. The broth/acetone filtrate was adjusted to pH 13 with 50% NaOH and refiltered through the ceramic filter (140 L filtrate) prior to loading onto a steel column (10 L; 10 cm X 122 cm) containing HP-20SS resin (Mitsubishi Chemical Industries, Ltd., Japan) at a flow rate of 1 L/minute. The column was eluted at a flow rate of 1 L/minute with a gradient mixed from solvent "A" (0.1% aq. NH_4OAc, adjusted to pH 8.1 with NH_4OH) and solvent "B" (CH_3CN - CH_3OH 1:1), collecting 4 L (approx.) fractions. The pumping system was programmed to generate a gradient from 0 to 50% B in one minute, followed by a gradient from 50 to 100% B in 90 minutes, followed by isocratic delivery of 100% B for an additional 10 minutes. HPLC analysis (see Example 7) indicated that pool 1 (fractions 16 - 21; 24.5 L), contained components H (12.32 g) and Q (0.34 g); pool 2 (fractions 22 - 25; 16L) contained components H (4.66 g), Q (2.06 g), R, S and T.

A. Isolation of pure component Q

Pool 2 was concentrated to dryness, redissolved in dichloromethane (50 ml), and applied to a glass column (5.5 cm x 30 cm) containing silica gel (EM grade 62, 60 - 200 mesh) equilibrated in dichloromethane. The column was washed with dichloromethane (3 L), then developed with dichloromethane - methanol (95.5), collecting 250 ml fractions. Fractions 3 through 15 were combined and concentrated to residue, then dissolved in ethanol/water (400 ml) and allowed to stand at room temperature over the weekend. The resulting crystals were washed with cold ethanol/water (1:1) and dried to give 6.1 g of dried crystals containing 68.7% component H and 31.2% component Q, by HPLC analysis.

The dried crystalline material was dissolved in tetrahydrofuran/methanol (1:1) and applied to a preparative reverse phase HPLC column (Rainin Dynamax-60A 8 µm C18, 41.4mm ID x 25 cm with 41.4 mm x 5 cm guard module) in 12 runs. The column was eluted at a flow rate of 50 ml/minute with a gradient mixed from solvent "A" (H_2O - CH_3CN; 30:35:35 containing 0.1% NH_4OAC) and solvent "B" (H_2O - CH_3CN - CH_3OH; 10:45:45 containing 0.1% NH_4OAC). The pumping system was programmed to generate a gradient from 50 to 100% B in 60 minutes. Progress of the separation was monitored with a variable wavelength UV detector tuned to 250 nm. Peak 1, containing component H (99%; 6 L) eluted first, followed by component Q. Combined peak 2 (containing component H, 20%, component Q, 80%; 8L) from all (12) runs was concentrated to 500 ml, reapplied to the same column, and eluted under the same mobile phase conditions in 5 runs. Pool 2 (2 L), containing 99% pure component Q was desalted by applying it to the same column equilibrated in H_2O - CH_3OH - CH_3CN (20:40:40).

The column was eluted with H_2O - CH_3OH - CH_3CN (10:45:45), collecting 10 three-minute fractions. Fractions 2 through 7 were combined, concentrated to residue, and dissolved in hot EtOH (80 ml). An equal volume of H_2O was added and the solution was allowed to cool overnight. The resulting crystals were collected on a filter, washed with cold EtOH - H_2O (1:1), and dried to give 1.5 g pure A83543Q.

B. Isolation of pure component R

Pool 1 from the HP-20SS chromatography was concentrated to residue and dissolved in methanol (200 ml). The component H- and Q-containing solution was precipitated into acetonitrile (3 L) and then filtered. The filtrate was concentrated to dryness, then dissolved in dichloromethane (100 ml) and applied to a glass column (5.5 cm x 30 cm) containing silica gel (EM grade 62, 60 - 200 mesh) equilibrated in dichloromethane. The column was washed with dichloromethane (2 L), then developed with dichloromethane - methanol (95:5), collecting 250 ml fractions. (Fractions 3 through 7, containing components S and T are discussed below, under isolation of pure components S and T.) Fractions 9 through 14 were combined and concentrated to residue, then dissolved in CH_3OH (10 ml) and applied to a preparative reverse phase HPLC column (Rainin Dynamax-60A 8 µm C18, 41.4 mm ID x 25 cm with 41.4 mm x 5 cm guard module) equilibrated in (H_2O - CH_3OH - CH_3CN; 30:35:35 containing 0.1% NH_4OAC containing 0.1% NH_4OAC) and solvent "B" (H_2O - CH_3CN - CH_3OH; 25:87.5:87.5 containing 0.1% NH_4OAC). The pumping system was programmed to generate a gradient from 0 to 100% B in 60 minutes. Progress of the separation was monitored with a variable wavelength UV detector tuned to 25 nm. The

fraction containing the major peak was desalted by applying it to the same column and eluting with a 60 minute gradient from H_2O - CH_3OH - CH_3CN (30:35:35) to H_2O - CH_3OH - CH_3CN (10:45:45), collecting 12 five-minute fractions. Fractions 2 through 12 were concentrated to residue, dissolved in t-BuOH, and lyophilized to give 1.77 g pure A83543R.

C. Isolation of pure components S and T

Fractions 3 through 7 from the dichloromethane-methanol (95:5) elution of the silica gel column (see Example 10, Section B) were combined, concentrated to residue, and dissolved in EtOH-H_2O (1:1 and allowed to stand at room temperature over the weekend). The resulting crystals were harvested by filtration and, washed with cold EtOH-H_2O (1:1), and dried to give 5.4 g of crystalline materials. The combined filtrate and wash were applied (in 10 runs) to a preparative reverse phase HPLC column (Rainin Dynamax-60A 8 µm C18, 41.4 mm ID x 25 cm with 41.4 mm x 5 cm guard module) equilibrated in (0.5% aq. NH_4OAc CH_3OH - CH_3CN; 20:40:40). The column was eluted at a flow rate of 40 ml/minute with a gradient mixed from solvent "A" (H_2O - CH_3OH - CH_3CN; 30:35:35 containing 0.1% NH_4OAC) and solvent "B" (H_2O - CH_3CN - CH_3OH; 10:45:45 containing 0.1% NH_4OAC). The pumping system was programmed to generate a gradient from 50 to 100% B in 60 minutes. Progress of the separation was monitored with a variable wavelength UV detector tuned to 250 nm; the major UV peak was collected in 4 pools. Pool 1 (5 L) contained component S; pool 5 (5 L), eluting ahead of the major UV peak, contained component T. The 5.4 g crystalline materials were dissolved, applied to the same column, and eluted under the same protocol to give pool 1 (5 L)

containing component T and pool 2 (2 L) containing component S, followed by components H and Q.

The component S-containing pools from the two reverse phase HPLC column (RP-HPLC) prep runs were combined, concentrated to residue, redissolved in 5 ml CH_3OH, and chromatographed on the same column with the same mobile phase gradient. Two UV absorbing (250 nm) peaks were collected. Peak 2 contained component H. Peak 1, containing component S was concentrated to 50 ml and applied to a Rainin Dynamax-60A 8µm C18, 21.4mm D x 25 cm with 21.4 mm x 5 cm guard module) equilibrated in H_2O - CH_3OH - CH_3CN (40:30:30). The column was eluted at a flow rate of 10 ml/minute with a gradient mixed from solvent "A" (H_2O - CH_3OH - CH_3CN; 40:30:30) and solvent "B" (H_2O - CH_3OH - CH_3CN; 10:45:45 with 0.1% NH_4OAc and 1% HOAc added). The pumping system was programmed to generate a gradient from 10 to 30% B in 60 minutes. The major UV absorbing peak was collected, concentrated to 1/2 volume, and desalted on the same HPLC column equilibrated in H_2O - CH_3OH - CH_3CN; 40:30:30, eluting with a 60 minute gradient from H_2O - CH_3OH - CH_3CN; (40:30:30) to H_2O - CH_3OH - CH_3CN (10:45:45), collecting 2 minute fractions. Fractions 2 through 8 were pooled and concentrated to dryness. The residue was dissolved in t-BuOH (5 ml) and lyophilized to give pure component S (182 mg).

The component T-containing pools from the two RP-HPLC prep runs were combined, concentrated to 100 ml, and applied (in 4 runs) to a preparative RP-HPLC column (Rainin Dynamax-60A 8 µm C18, 21.4 mm ID x 25 cm with 21.4 m x 5 cm guard module) equilibrated in 0.33% aq. NH_4OAc - CH_3OH - CH_3CN (30:35:35). The column was eluted at a flow rate of 10 ml/minute with a gradient

mixed from solvent "A" (H_2O-CH_3OH - CH_3CN; 30:35:35 containing 0.1% NH_4OAC) and solvent "B" (H_2O - CH_3OH-CH_3CN; 10:45:45 containing 0.1% NH_4OAC). The pumping system was programmed to generate a gradient from 25 to 75% B in 60 minutes. One peak contained pure component R. The other peak, containing a mixture of components R and T, was rechromatographed under the same conditions. The pure component T containing peak from the latter HPLC preparative run was desalted on the same column equilibrated in H_2O - CH_3OH - CH_3CN (30:35:35) and eluted with H_2O - CH_3OH - CH_3CN (10:45:45). The UV absorbing peak was concentrated to dryness. The residue was dissolved in t-BuOH and lyophilized to give pure component T (166 mg).

Example 11

Preparation of Compound 8 (N-demethyl A83543D)

A. Isolation of A83543A and A83543D

Components A83543A and D were isolated essentially according to the teachings of examples 2-4 of EPO 0 375 316 A1.

B. Synthesis of A83543B and N-demethyl A83543D

A suspension, with 75% purity, of A83543 components A and D (85:15 mixture, respectively) was prepared. Millimolar amount was based on the molecular weight of A83543A.

The suspension of A83543 (5.0 grams, 5.13 mmol) and sodium acetate tri-hydrate (4.68 grams, 34.4 millimoles) in 80% methanol/water (125 ml) was heated to 47°C under nitrogen. The pH dropped from 10 to 8 on

addition of iodine (1.75 gms, 68.0 mmol) as a solid in one portion, giving a brown color. The pH was maintained at 8-9 by periodic addition of 1N sodium hydroxide. The reaction was heated for 2.75 hours (during which time the color faded to pale yellow), and was then cooled to ambient temperature. The solution was poured into a solution of water (250 ml) and ammonium hydroxide (50 ml), extracted with diethyl ether. The ether extract was washed with brine, dried with potassium carbonate (K_2CO_3), and evaporated at ambient temperature under vacuum.

The crude product is purified by reversed phase HPLC on a C_{18} column, eluting with methanol: acetonitrile: 0.05% ammonium acetate (45:45:10) giving two products. The more polar product (2.52 gms) was identical (MS, ^1H NMR, ^{13}C NMR, IR, and OR) to an authentic sample of A83543B. The less polar product (202149; 206 mg) is found to be mono-N-demethyl-83543D: H NMR (270 MHz, acetone-d); ^{13}C NMR (270 MHz, acetone-d_6); IR ($CHCl_3$) 3200-2800 (br), 1720, 1660, 1620 cm^{-1}; MS (FD) m/z 1485 (dimer+Na, 60), 1464 (dimer, 30), 1463 (10), 733 (M^+, 100), 731 (90); UV (EtOH) λ_{max} 244 nm (ε9400).

Example 12

Preparation A83543AgA (Compound 17)

To a solution of A83543PsaA1 (6.03 g, 10.7 mmol) in methanol (267 ml), 7.2N H_2SO_4 (396 ml) was added, and the solution was heated to reflux for 3 hours. The mixture was then cooled in an ice bath. A large amount of $NaHCO_3$ (solid) and saturated aqueous $NaHCO_3$ were added cautiously; however, the pH was never

brought above 1.0. The aqueous solution was mixed with ethyl ether and separated. The aqueous portion was then extracted with fresh ethyl ether. The ether extracts were combined, washed with brine, dried with K_2CO_3, and evaporated at reduced pressure. The resulting yellow semi-solid (4.89 g) was purified by normal phase chromatography with 100% dichloromethane and a gradient up to 7.5 percent methanol in dichloromethane, giving A83543AgA (2.83 g, 66 percent yield) as a colorless glass.

Example 13

Preparation of Compound 9

A suspension of N-chlorosuccinimide (104.7 mg, 0.78 mmol) in dichloromethane (2.6 ml) was cooled to -78°C under nitrogen. Diisopropyl sulfide (0.125 ml, 0.86 mmol) was added to this suspension, and the mixture was stirred at -78°C for one half hour. A83543J (184.6 mg, 0.26 mmol) in dichloromethane (1 ml) was then added slowly. When the addition was completed, the solution was stirred at -78°C for 6.25 hours. Triethylamine (0.109 ml, 0.78 mmol) was then added, and the solution was warmed to room temperature. The mixture was red. After warming, ethyl ether (6 ml) was added and a precipitate formed. The precipitate was dissolved in dichloromethane, and this was combined with the ethyl ether solution. The resulting solution was washed with 0.1N HCl, then washed with brine, dried with $MgSO_4$, and evaporated at room temperature. The resulting colorless glass (215 mg) was semi-purified by flash chromatography with 5 percent methanol in dichloromethane, giving Compound 9 as a colorless semi-solid (151.2 mg). The weight recovery and NMR spectrum showed contamination of

product with diisopropyl sulfide, but the product was used without further purification.

Example 14

Preparation of Compound 10

The procedure used in Example 13 was repeated starting with A83543L (997.4 mg, 1.36 mmol), and gave Compound 10 as a colorless semi-solid (850 mg).

Example 15

Preparation of A83543PsaA2 (Compound 13)

To a solution of Compound 9 (1.89 g, 2.64 mmol) in methanol (100 ml), K_2CO_3 (anhydrous; 1.82 g, 13.2 mmol) was added, and the mixture was stirred at room temperature for one hour. Ethyl ether (100 ml) was then added and the mixture was filtered. The filtrate was evaporated at room temperature, giving a yellow solid. The yellow solid was dissolved in dichloromethane, washed with water, then brine, and dried with $MgSO_4$. The dichloromethane was then evaporated at reduced pressure, giving a colorless semi-solid (1.53 g). This semi-solid was purified by flash chromatography with 5 percent methanol in dichloromethane to 10 percent methanol in dichloromethane in a one-step gradient, giving A83543PsaA2 (1.09 g, 76 percent yield) as an off white glass.

Example 16

Preparation of A83543PsaD2 (Compound 14)

The procedure described in Example 15 was repeated using as starting material the product of

Example 13 (770 mg, 1.06 mmol) and producing A83543PsaD2 (246 mg, 42 percent yield) as a colorless glass.

Example 17

Preparation of A83543AgD (Compound 18)

To a suspension of A83543PsaD2 (132 mg, 0.24 mmol) in water (5 ml), 1N H_2SO_4 was added dropwise until the mixture had a pH of 1.7 and was homogeneous. This solution was heated to 80°C for 3.75 hours, during which time an oil separated from the solution. The mixture was cooled to room temperature and dichloromethane was added to dissolve the oil. The aqueous layer was separated and extracted with fresh dichloromethane. The dichloromethane solutions were combined, washed quickly with 1N H_2SO_4, dried with K_2CO_3, and evaporated at room temperature giving a pale yellow glass (82.9 mg). The product was purified by flash chromatography with 5 percent methanol in dichloromethane, giving A83543AgD (63.6 mg, 63 percent yield) as a colorless glass.

Compounds 21 and 22 can be prepared by chemical demethylation of compounds 13 and 14, respectively, using sodium methoxide/iodine. The reaction is preferably carried out in a polar organic solvent, such as methanol. Further, the reaction is carried out at a temperature from about -10°C to about 15°C, preferably between 0°C and 5°C. The reaction times vary from about 4 hours to about 6 hours.

Example 18

Preparation of A83543PsaL1 (Compound 23)

A sample of A83543L (1.0 g) was added to deionized water (90 ml) and a sufficient volume of 1N

H_2SO_4 (approximately 0.5 ml) was added to cause dissolution. This solution was heated at about 80°C for 2 hours, and the resulting mixture was allowed to cool to room temperature. The precipitate was collected by filtration, washed with cold deionized water, and dried to give 420 mg of impure A83543PsaL1. The aqueous washes were combined, saturated with NaCl, and extracted with methylene chloride. The combined methylene chloride extracts were washed with brine, dried (K_2CO_3), and evaporated to dryness to give 368 mg of a white glass. The residual glass was combined with the precipitate and purified by flash chromatography (silica gel 60, 230-400 mesh), eluting with a mixture of ethyl acetate and hexane (7:3). The fractions containing the desired compound were combined and evaporated to dryness to give 382.8 mg of A83543PsaL1 as a colorless glass.

MS (FD): m/z 590, 591 (M+), 592 (M+H)
UV (EtOH): λ_{max} 242 nm (e = 10,048)

Example 19

The compounds of this invention are useful as intermediates in the preparation of insecticides. For example, when appropriate microorganisms are cultured in the presence of the claimed compounds, the claimed compounds are bioconverted to insecticidally active A83543 components, as illustrated below.

This example illustrates the preparation of A83543A by culturing NRRL 18538 in the presence of A83543AgA. The culture *Saccharopolyspora spinosa* NRRL 18538, either as a lyophilized pellet or as a suspension maintained in liquid nitrogen, was used to inoculate a vegetative medium having the following composition:

Ingredient	Amount (g)
Enzyme-hydrolyzed casein	30
Yeast extract	3
$MgSO_4 \cdot 7H_2O$	2
Glucose	10
Deionized water	q.s. to 1 L

The pH was adjusted to 6.5 with sodium hydroxide.

Slants or plates can be prepared by adding 2.5% agar to the vegetative medium. The inoculated slant is incubated at 30°C for 10 to 14 days. The mature slant culture is scraped with a sterile tool to loosen the spores and remove and macerate the mycelial mat. About one-fourth of the loosened spores and culture growth thus obtained is used to inoculate 50 ml of a first-stage vegetative medium. Alternatively, the first-stage medium may be inoculated from a liquid nitrogen ampoule.

When the culture is maintained in liquid nitrogen, ampoules are prepared using equal volumes of vegetative culture (48-72 hours incubation, 30°C) and suspending medium. The suspending medium contains lactose (100 g), glycerol (200 ml), and deionized water (q.s. to 1 L).

A liquid nitrogen ampoule is used to inoculate 100 ml of vegetative medium in 500 ml Erlenmeyer flasks (or 50 ml of medium in 250 ml flasks). The cultures are incubated at 30°C for 48 hours on a shaker orbiting in a two inch (5.08 cm) circle at 250 rpm. The incubated culture (5% v/v inoculum) is used to inoculate 100 ml of a production medium having the following composition:

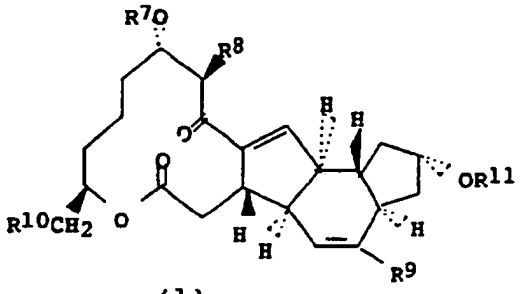
Ingredient	Amount (g)
Glucose	80
Peptionized milk	20
Cottonseed flour	30
Corn steep liquor	10
Ca_2CO_3	5
Methyl oleate	30
Tap water	q.s. to 1L

The pH was adjusted to 7.2 with sodium hydroxide.

Conversion of A83543AgA to A83543A was accomplished by addition of A83543AgA (4.88 mg, 0.195 mg/ml) to a 65 hr culture of NRRL 18538 in the above-mentioned production medium (25 ml in a 250 ml flask) and incubating the culture for an additional 31 hours. Acetonitrile (3.0 ml) was added to an aliquot (1.0 ml) of the culture. This sample was mixed and centrifuged and an aliquot was injected onto an analytical HPLC column designed to assay the various components of the A83543 culture. Analysis of the fermentation broth showed the presence of 2.44 mg (0.098 mg/ml) of A83543A.

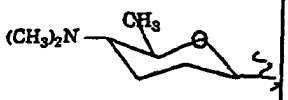
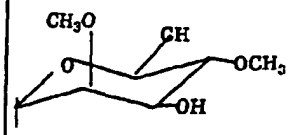
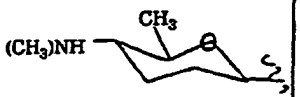
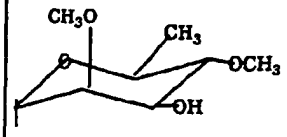
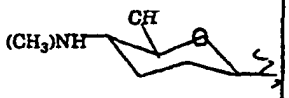
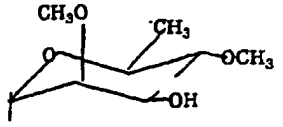
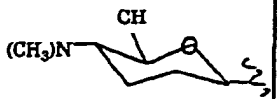
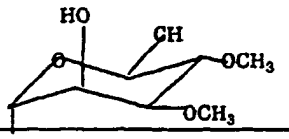
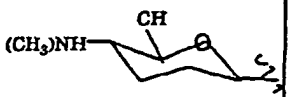
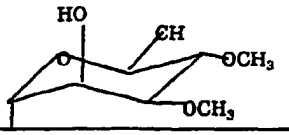
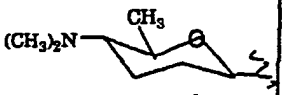
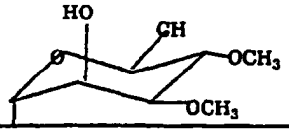
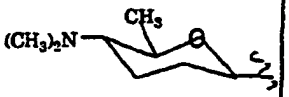
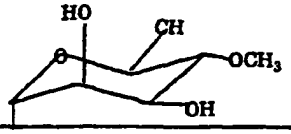
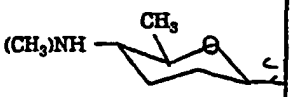
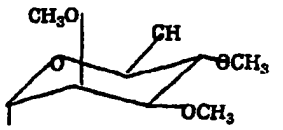
WHAT IS CLAIMED IS:

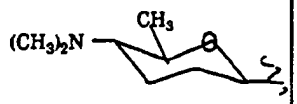
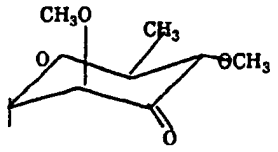
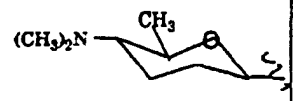
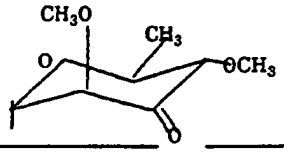
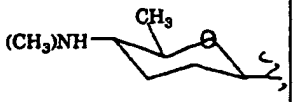
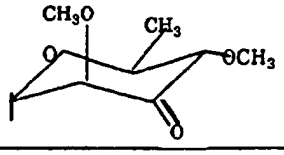
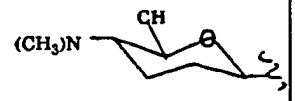
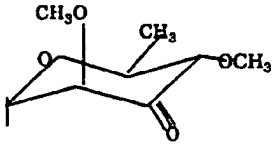
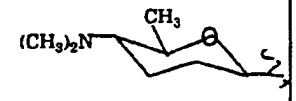
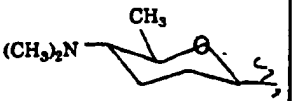
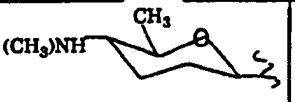
1. A compound of the Formula 1:

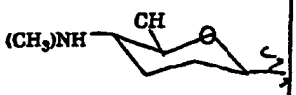
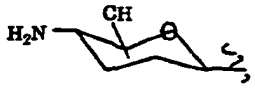
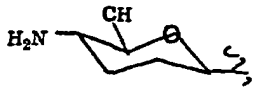
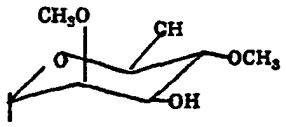


(1)

wherein R^7, R^8, R^9 and R^{10} are each individually as follows:

Compound	R^7	R^8	R^9	R^{10}	R^{11}
Compound 1 (A83543L)		Me	Me	Me	
Compound 2 (A83543M)		Me	H	Me	
Compound 3 (A83543N)		Me	Me	Me	
Compound 4 (A83543Q)		Me	Me	Me	
Compound 5 (A83543R)		Me	H	Me	
Compound 6 (A83543S)		Me	H	H	
Compound 7 (A83543T)		Me	H	Me	
Compound 8 (N-demethyl A83543D)		Me	Me	Me	

Compound	R^7	R^8	R^9	R^{10}	R^{11}
Compound 9		Me	H	Me	
Compound 10		Me	Me	Me	
Compound 11		Me	H	Me	
Compound 12		Me	Me	Me	
Compound 13 (A83543 PsaA2)		Me	H	Me	H
Compound 14 (A83543 PsaD2)		Me	Me	Me	H
Compound 15 (A83543 PsaB2)		Me	H	Me	H

Compound	R^7	R^8	R^9	R^{10}	R^{11}
Compound 16 (A83543 PsaN2)		Me	Me	Me	H
Compound 17 (A83543 AgA)	H	Me	H	Me	H
Compound 18 (A83543 AgD)	H	Me	Me	Me	H
Compound 19 (A83543 AgE)	H	Me	H	H	H
Compound 20 (A83543 AgF)	H	H	H	Me	H
Compound 21 (A83543 PsaC2)		Me	H	Me	H
Compound 22		Me	Me	Me	H
Compound 23 (A83543 PsaL2)	H	Me	Me	Me	

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2. A process for preparing A83534J, A83543L, A843543M and A84543N which comprises cultivating an A83543J-producing mutant of *Saccharopolyspora spinosa* strain NRRL 18395, in a suitable culture medium, under submerged aerobic fermentation conditions until a recoverable amount of the compound is produced.

3. The process of Claim 2 further comprising the step of separating A83543J.

4. The process of Claim 2 further comprising the step of separating A83543L.

5. The process of Claim 2 further comprising the step of separating A83543M.

6. The process of Claim 2 further comprising the step of separating A83543N.

7. The process of Claim 2 wherein the A83543J-producing strain of *S. spinosa* is NRRL 18719, or an A83543J-producing mutant thereof.

8. The process of Claim 2 wherein the A83543J-producing strain of *S. spinosa* is NRRL 18720, or an A83543J-producing mutant thereof.

9. A process for preparing A83534Q, A83543R, A843543S and A84543T which comprises cultivating an A83543Q-producing mutant of *S. spinosa* strain NRRL 18395, in a suitable culture medium, under submerged aerobic fermentation conditions until a recoverable amount of the compound is produced.

10. The process of Claim 9 further comprising the step of separating component A83543Q.

11. The process of Claim 9 further comprising the step of separating component A83543R.

12. The process of Claim 9 further comprising the step of separating component A83543S.

13. The process of Claim 9 further comprising the step of separating A83543T.

14. The process of Claim 9 wherein the A83543Q-producing strain of *S. spinosa* is NRRL 18723, or an A83543Q-producing mutant thereof.

15. A process for preparing N-demethyl A83543D which comprises the step of reacting A83543D with iodine in the presence of an inert solvent and a weak base selected from the group consisting of sodium acetate, propionate and benzoate.

16. An insecticide or miticide composition comprising a phytologically-acceptable carrier in combination with an insect-inactivating amount of at least one compound selected from one of A83543L, A83543M, A83543N, A83543Q, A83543R, A83543S, A83543T and N-demethyl A83543D.

17. An insecticide or miticide method which comprises applying to the locus of an insect or mite an insect- or mite-inactivating amount of at least one compound selected from one of A83543L, A83543M, A83543N, A83543Q, A83543R, A83543S, A83543T and N-demethyl A83543D.

18. An ectoparasiticidal composition comprising a physiologically-acceptable inert carrier and at least one compound selected from one of A83543L,

A83543M, A83543N, A83543Q, A83543R, A83543S, A83543T and N-demethyl A83543D.

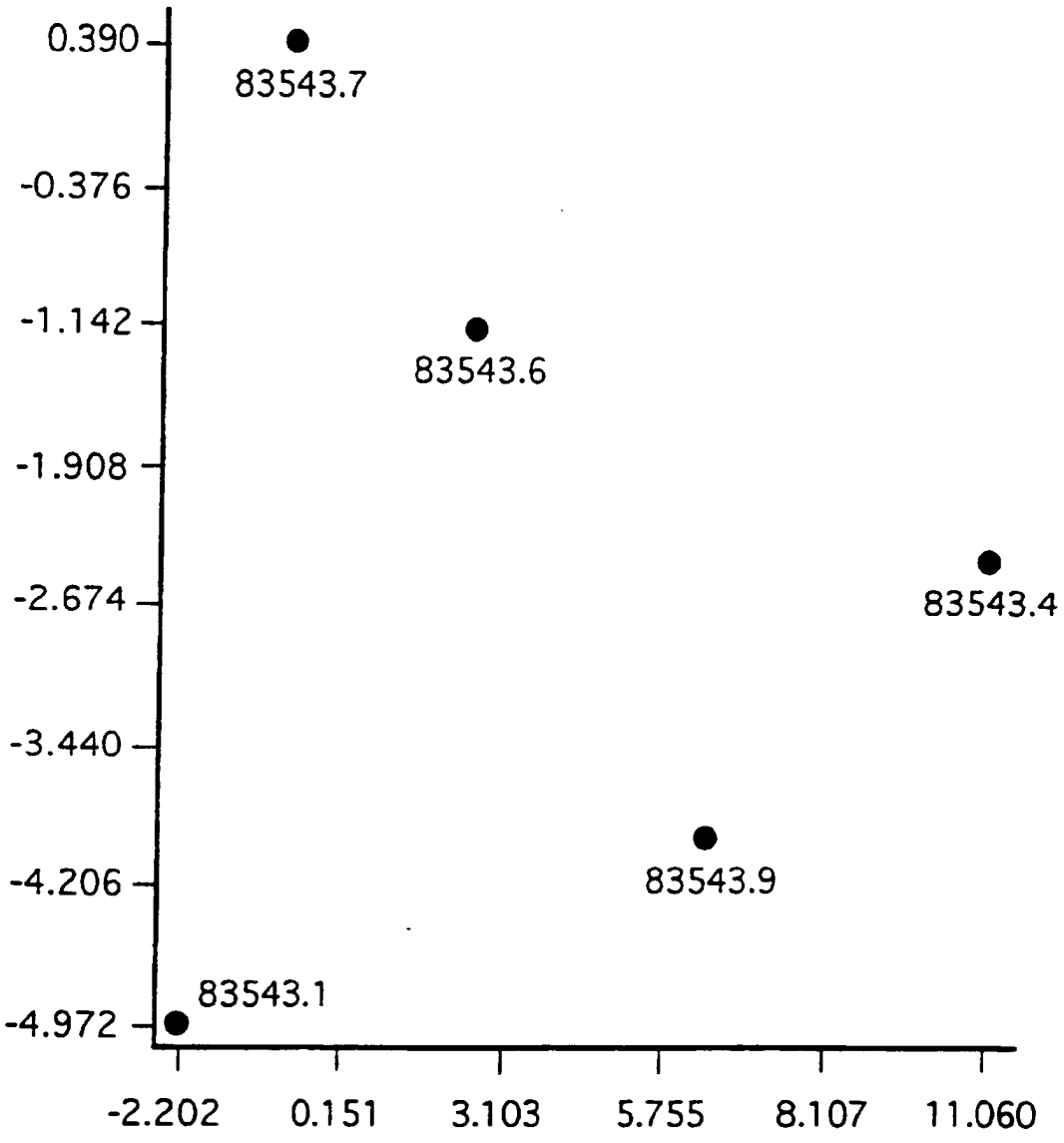
19. A method of controlling a population of insect ectoparasites which consume blood of a host animal which comprises administering to the host animals at least one compound selected from one of A83543L, A83543M, A83543N, A83543Q, A83543R, A83543S, A83543T and N-demethyl A83543D.

20. A biologically pure culture of *Saccharopolyspora spinosa* NRRL 18719, or an A83543J-producing mutant thereof.

21. A biologically pure culture of *Saccharopolyspora spinosa* NRRL 18720, or an A83543J-producing mutant thereof.

22. A biologically pure culture of *Saccharopolyspora spinosa* NRRL 18823, or an A83543Q-producing mutant thereof.

FIG. 1



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/09684

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07H17/08; C12P19/62; C12N1/20; A01N43/22
//(C12N1/20,C12R:01)(C12P19/62,C12R1:01)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07H ; C12P ; C12N ; A01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP,A,0 375 316 (ELI LILLY AND COMPANY) 27 June 1990 cited in the application	1,16-19
A	see the whole document	2-15, 20-22

P,X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY. vol. 114, no. 6, 11 March 1992, GASTON, PA US pages 2260 - 2 D.A.EVANS ET AL. 'Asymmetric Synthesis of the Macrolide (+)-A83543A (Lepicidin) Aglycon.' see the whole document	1,16-19
	-----	

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 FEBRUARY 1993	24.03.93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCOTT J.R.

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9209684
SA 67883

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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